

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
29 August 2002 (29.08.2002)

PCT

(10) International Publication Number
WO 02/066627 A1

(51) International Patent Classification⁷: C12N 9/48, 15/12, 5/10, C12Q 1/68, G01N 33/50, C12N 15/62

(21) International Application Number: PCT/EP02/01538

(22) International Filing Date: 14 February 2002 (14.02.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/268,863 16 February 2001 (16.02.2001) US

(71) Applicant (for all designated States except US): BAYER AKTIENGESELLSCHAFT [DE/DE]; 51368 Leverkusen (DE).

(72) Inventor; and

(75) Inventor/Applicant (for US only): LIOU, JingRen [CN/US]; 10 Winslow Road, Belmont, MA 02478 (US).

(74) Common Representative: BAYER AKTIENGESELLSCHAFT; 51368 Leverkusen (DE).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(i)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/066627 A1

(54) Title: REGULATION OF HUMAN DIPEPTIDYL PEPTIDASE 8

(57) Abstract: Reagents that regulate human dipeptidyl peptidase 8 and reagents which bind to human dipeptidyl peptidase 8 gene products can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, cancer, CNS disorders, and COPD.

REGULATION OF HUMAN DIPEPTIDYL PEPTIDASE 8

TECHNICAL FIELD OF THE INVENTION

5

The invention relates to the regulation of human dipeptidyl peptidase 8.

BACKGROUND OF THE INVENTION

10 Dipeptidyl peptidase is responsible for the removal of terminal dipeptides sequentially from polypeptides. There is a need in the art to identify related enzymes, which can be regulated to provide therapeutic effects.

SUMMARY OF THE INVENTION

15

It is an object of the invention to provide reagents and methods of regulating a human dipeptidyl peptidase 8. This and other objects of the invention are provided by one or more of the embodiments described below.

20 One embodiment of the invention is a dipeptidyl peptidase 8 polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 62% identical to the amino acid sequence shown in SEQ ID NO: 2 and;

25 the amino acid sequence shown in SEQ ID NO: 2;

Yet another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a dipeptidyl peptidase 8 polypeptide comprising an amino acid sequence selected from the group consisting of:

30

amino acid sequences which are at least about 62% identical to the amino acid sequence shown in SEQ ID NO: 2 and; the amino acid sequence shown in SEQ ID NO: 2;

5 Binding between the test compound and the dipeptidyl peptidase 8 polypeptide is detected. A test compound which binds to the dipeptidyl peptidase 8 polypeptide is thereby identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the activity of the dipeptidyl peptidase 8.

10 Another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a polynucleotide encoding a dipeptidyl peptidase 8 polypeptide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

15 nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1 and; the nucleotide sequence shown in SEQ ID NO: 1;

20 Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the amount of the dipeptidyl peptidase 8 through interacting with the dipeptidyl peptidase 8 mRNA.

25 Another embodiment of the invention is a method of screening for agents which regulate extracellular matrix degradation. A test compound is contacted with a dipeptidyl peptidase 8 polypeptide comprising an amino acid sequence selected from the group consisting of:

30 amino acid sequences which are at least about 62% identical to the amino acid sequence shown in SEQ ID NO: 2 and; the amino acid sequence shown in SEQ ID NO: 2;

A dipeptidyl peptidase 8 activity of the polypeptide is detected. A test compound which increases dipeptidyl peptidase 8 activity of the polypeptide relative to dipeptidyl peptidase 8 activity in the absence of the test compound is thereby identified

5 as a potential agent for increasing extracellular matrix degradation. A test compound which decreases dipeptidyl peptidase 8 activity of the polypeptide relative to dipeptidyl peptidase 8 activity in the absence of the test compound is thereby identified as a potential agent for decreasing extracellular matrix degradation.

10 Even another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a dipeptidyl peptidase 8 product of a polynucleotide which comprises a nucleotide sequence selected from the group consisting of:

15 nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1 and; the nucleotide sequence shown in SEQ ID NO: 1;

20 Binding of the test compound to the dipeptidyl peptidase 8 product is detected. A test compound which binds to the dipeptidyl peptidase 8 product is thereby identified as a potential agent for decreasing extracellular matrix degradation.

25 Still another embodiment of the invention is a method of reducing extracellular matrix degradation. A cell is contacted with a reagent which specifically binds to a polynucleotide encoding a dipeptidyl peptidase 8 polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

30 nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1 and; the nucleotide sequence shown in SEQ ID NO: 1;

Dipeptidyl peptidase 8 activity in the cell is thereby decreased.

5 The invention thus provides a human dipeptidyl peptidase 8 that can be used to identify test compounds that may act, for example, as activators or inhibitors at the enzyme's active site. Human dipeptidyl peptidase 8 and fragments thereof also are useful in raising specific antibodies that can block the enzyme and effectively reduce its activity.

10 **BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 1).

15 Fig. 2 shows the amino acid sequence deduced from the DNA-sequence of Fig.1 (SEQ ID NO: 2).

Fig. 3 shows the amino acid sequence of a protein identified by trembl|AF221634|AF221634_1 (SEQ ID NO: 3).

20 Fig. 4 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 4).

Fig. 5 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 5).

25 Fig. 6 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 6).

30 Fig. 7 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 7).

Fig. 8 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 8).

5 Fig. 9 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 9).

Fig. 10 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 10).

10 Fig. 11 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 11).

Fig. 12 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 12).

15 Fig. 13 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 13).

20 Fig. 14 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 14).

Fig. 15 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 15).

25 Fig. 16 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 16).

Fig. 17 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 17).

30

- 6 -

Fig. 18 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 18).

5 Fig. 19 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 19).

Fig. 20 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 20).

10 Fig. 21 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 21).

Fig. 22 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 22).

15 Fig. 23 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 23).

Fig. 24 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 24).

20 Fig. 25 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 25).

25 Fig. 26 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 26).

Fig. 27 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 27).

30

Fig. 28 shows the DNA-sequence encoding a dipeptidyl peptidase 8 Polypeptide (SEQ ID NO: 28).

5 Fig. 29 shows the BLASTP - alignment of 181_Protein (SEQ ID NO: 2) against trembl|AF221634|AF221634_1 (SEQ ID NO: 3).

Fig. 30 shows the HMMPFAM - alignment of 181_Protein (SEQ ID NO: 2) against pfam|hmm|DPPIV_N_term.

10 Fig. 31 shows the HMMPFAM - alignment of 181_Protein (SEQ ID NO: 2) against pfam|hmm|Peptidase_S9.

DETAILED DESCRIPTION OF THE INVENTION

15 The invention relates to an isolated polynucleotide from the group consisting of:

a) a polynucleotide encoding a dipeptidyl peptidase 8 polypeptide comprising an amino acid sequence selected from the group consisting of:

20 amino acid sequences which are at least about 62% identical to the amino acid sequence shown in SEQ ID NO: 2 and; the amino acid sequence shown in SEQ ID NO: 2;

b) a polynucleotide comprising the sequence of SEQ ID NO: 1;

25 c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b) and encodes a dipeptidyl peptidase 8 polypeptide;

- d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code and encodes a dipeptidyl peptidase 8 polypeptide; and
- 5 e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d) and encodes a dipeptidyl peptidase 8 polypeptide.

10 Furthermore, it has been discovered by the present applicant that a novel dipeptidyl peptidase 8, particularly a human dipeptidyl peptidase 8, can be used in therapeutic methods to treat cancer, a CNS disorder or COPD.

15 Human dipeptidyl peptidase 8 comprises the amino acid sequence shown in SEQ ID NO: 2. A coding sequence for human dipeptidyl peptidase 8 is shown in SEQ ID NO: 1. This sequence is located on chromosome 19. Related ESTs (SEQ ID NOS: 4-28) are expressed in colon; muscle (rhabdomyosarcoma); placenta (chorio-carcinoma); nervous_tumor; skin (melanotic melanoma); bone marrow (acute myelogenous leukemia); lung (small cell carcinoma); uterus_tumor; breast; marrow; lung_normal; adrenal gland (adrenal adenoma); and head_neck.

20 Human dipeptidyl peptidase 8 is 61% identical over 840 amino acids to trembl|AF221634|AF221634_1 (SEQ ID NO: 3) (FIG. 1). Both pfam and blocks searches confirm this protein's peptidase function based on its homology to dipeptidyl peptidase and prolyl oligopeptidase. In addition, the critical active site serine, aspartate, and histidine residues are found in the molecule.

25 Human dipeptidyl peptidase 8 of the invention is expected to be useful for the same purposes as previously identified dipeptidyl peptidase 8 enzymes. Human dipeptidyl peptidase 8 is believed to be useful in therapeutic methods to treat disorders such as cancer, CNS disorders, and COPD. Human dipeptidyl peptidase 8 also can be used to screen for human dipeptidyl peptidase 8 activators and inhibitors.

Polypeptides

Human dipeptidyl peptidase 8 polypeptides according to the invention comprise at least 6, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, or 892 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO: 2 or a biologically active variant thereof, as defined below. A dipeptidyl peptidase 8 polypeptide of the invention therefore can be a portion of a dipeptidyl peptidase 8 protein, a full-length dipeptidyl peptidase 8 protein, or a fusion protein comprising all or a portion of a dipeptidyl peptidase 8 protein.

Biologically Active Variants

Human dipeptidyl peptidase 8 polypeptide variants that are biologically active, e.g., retain a dipeptidyl peptidase activity, also are dipeptidyl peptidase 8 polypeptides. Preferably, naturally or non-naturally occurring dipeptidyl peptidase 8 polypeptide variants have amino acid sequences which are at least about 62, 65, or 70, preferably about 75, 80, 85, 90, 96, 96, 98, or 99% identical to the amino acid sequence shown in SEQ ID NO: 2 or a fragment thereof. Percent identity between a putative dipeptidyl peptidase 8 polypeptide variant and an amino acid sequence of SEQ ID NO: 2 is determined using the Blast2 alignment program (Blosum62, Expect 10, standard genetic codes).

Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

- 10 -

Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of a dipeptidyl peptidase 8 polypeptide can be 5 found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active dipeptidyl peptidase 8 polypeptide can readily be determined by assaying for dipeptidyl peptidase activity, as described for example, in Maes *et al.*, *Neuropsychopharmacology*. 2001 Feb;24(2):130-40; Sentandreu & Toldra, *J Agric Food Chem*. 2000 Oct;48(10):5014-10 22; Li *et al.*, *Biochem Biophys Res Commun*. 2000 Sep 24;276(2):553-8; or Durinx *et al.*, *Eur J Biochem*. 2000 Sep;267(17):5608-13.

Fusion Proteins

15 Fusion proteins are useful for generating antibodies against dipeptidyl peptidase 8 polypeptide amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins that interact with portions of a dipeptidyl peptidase 8 polypeptide. Protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage 20 display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

25 A dipeptidyl peptidase 8 polypeptide fusion protein comprises two polypeptide segments fused together by means of a peptide bond. The first polypeptide segment comprises at least 6, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, or 892 contiguous amino acids of SEQ ID NO: 2 or of a biologically active variant, such as those described above. The first polypeptide segment also can comprise full-length dipeptidyl peptidase 8 protein.

The second polypeptide segment can be a full-length protein or a protein fragment. Proteins commonly used in fusion protein construction include β -galactosidase, β -glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horse-
5 radish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA
10 binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between the dipeptidyl peptidase 8 polypeptide-encoding sequence and the heterologous protein sequence, so that the dipeptidyl peptidase 8 polypeptide can be cleaved and purified away from the heterologous moiety.

15

A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two polypeptide segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which
20 comprises coding sequences selected from SEQ ID NO: 1 in proper reading frame with nucleotides encoding the second polypeptide segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Identification of Species Homologs

30 Species homologs of human dipeptidyl peptidase 8 polypeptide can be obtained using dipeptidyl peptidase 8 polypeptide polynucleotides (described below) to make

suitable probes or primers for screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of dipeptidyl peptidase 8 polypeptide, and expressing the cDNAs as is known in the art.

5

Polynucleotides

10 A dipeptidyl peptidase 8 polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for a dipeptidyl peptidase 8 polypeptide. A coding sequence for human dipeptidyl peptidase 8 is shown in SEQ ID NO: 1.

15 Degenerate nucleotide sequences encoding human dipeptidyl peptidase 8 polypeptides, as well as homologous nucleotide sequences which are at least about 50, 55, 60, 65, 70, preferably about 75, 90, 96, 98, or 99% identical to the nucleotide sequence shown in SEQ ID NO: 1 or its complement also are dipeptidyl peptidase 8 polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and 20 a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of dipeptidyl peptidase 8 polynucleotides that encode biologically active dipeptidyl peptidase 8 polypeptides also are dipeptidyl peptidase 8 polynucleotides. Polynucleotide fragments comprising at least 8, 9, 10, 11, 12, 15, 25 20, or 25 contiguous nucleotides of SEQ ID NO: 1 or its complement also are dipeptidyl peptidase 8 polynucleotides. These fragments can be used, for example, as hybridization probes or as antisense oligonucleotides.

Identification of Polynucleotide Variants and Homologs

30 Variants and homologs of the dipeptidyl peptidase 8 polynucleotides described above also are dipeptidyl peptidase 8 polynucleotides. Typically, homologous dipeptidyl

peptidase 8 polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known dipeptidyl peptidase 8 polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50°C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

10

Species homologs of the dipeptidyl peptidase 8 polynucleotides disclosed herein also can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of dipeptidyl peptidase 8 polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5°C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol.* 81, 123 (1973)). Variants of human dipeptidyl peptidase 8 polynucleotides or dipeptidyl peptidase 8 polynucleotides of other species can therefore be identified by hybridizing a putative homologous dipeptidyl peptidase 8 polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NO: 1 or the complement thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

25

Nucleotide sequences which hybridize to dipeptidyl peptidase 8 polynucleotides or their complements following stringent hybridization and/or wash conditions also are dipeptidyl peptidase 8 polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

- 14 -

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20°C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a dipeptidyl peptidase 8 polynucleotide having a nucleotide sequence shown in SEQ ID NO: 1 or 5 the complement thereof and a polynucleotide sequence which is at least about 50, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A.* 48, 1390 (1962):

10
$$T_m = 81.5^\circ\text{C} - 16.6(\log_{10}[\text{Na}^+]) + 0.41(\%G + C) - 0.63(\%\text{formamide}) - 600/l,$$
 where l = the length of the hybrid in basepairs.

15 Stringent wash conditions include, for example, 4X SSC at 65°C, or 50% formamide, 4X SSC at 42°C, or 0.5X SSC, 0.1% SDS at 65°C. Highly stringent wash conditions include, for example, 0.2X SSC at 65°C.

Preparation of Polynucleotides

20 A dipeptidyl peptidase 8 polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated dipeptidyl peptidase 8 polynucleotides. For 25 example, restriction enzymes and probes can be used to isolate polynucleotide fragments, which comprise dipeptidyl peptidase 8 nucleotide sequences. Isolated polynucleotides are in preparations that are free or at least 70, 80, or 90% free of other molecules.

- 15 -

Human dipeptidyl peptidase 8 cDNA molecules can be made with standard molecular biology techniques, using dipeptidyl peptidase 8 mRNA as a template. Human dipeptidyl peptidase 8 cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as 5 Sambrook *et al.* (1989). An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention, using either human genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesize dipeptidyl peptidase 8 polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a dipeptidyl peptidase 8 polypeptide having, for example, an amino acid sequence shown in SEQ ID NO: 2 or 10 a biologically active variant thereof.

15 Extending Polynucleotides

Various PCR-based methods can be used to extend the nucleic acid sequences disclosed herein to detect upstream sequences such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to retrieve 20 unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic.* 2, 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are 25 transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al.*, *Nucleic Acids Res.* 16, 8186, 1988). Primers 30 can be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30

nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

5

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic.* 1, 111-119, 1991). In this method, multiple restriction enzyme digestions and ligations also can be used to 10 place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res.* 19, 3055-3060, 1991). Additionally, PCR, nested primers, 15 and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA (CLONTECH, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been 20 size-selected to include larger cDNAs. Randomly-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

25

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) that are laser activated, and detection of the emitted wavelengths by a charge coupled device 30 camera. Output/light intensity can be converted to electrical signal using appropriate

software (e.g. GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA that might be present in limited amounts

5 in a particular sample.

Obtaining Polypeptides

10 Human dipeptidyl peptidase 8 polypeptides can be obtained, for example, by purification from human cells, by expression of dipeptidyl peptidase 8 polynucleotides, or by direct chemical synthesis.

Protein Purification

15 Human dipeptidyl peptidase 8 polypeptides can be purified from any cell that expresses the polypeptide, including host cells that have been transfected with dipeptidyl peptidase 8 expression constructs. A purified dipeptidyl peptidase 8 polypeptide is separated from other compounds that normally associate with the dipeptidyl peptidase 8 polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis. A preparation of purified dipeptidyl peptidase 8 polypeptides is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-20 polyacrylamide gel electrophoresis.

25

Expression of Polynucleotides

30 To express a dipeptidyl peptidase 8 polynucleotide, the polynucleotide can be inserted into an expression vector that contains the necessary elements for the

transcription and translation of the inserted coding sequence. Methods that are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding dipeptidyl peptidase 8 polypeptides and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1989.

10 A variety of expression vector/host systems can be utilized to contain and express sequences encoding a dipeptidyl peptidase 8 polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (e.g., baculovirus), plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids), or animal cell systems.

15

20 The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO, and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are

25

30

preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding a dipeptidyl peptidase 8 polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

5 *Bacterial and Yeast Expression Systems*

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the dipeptidyl peptidase 8 polypeptide. For example, when a large quantity of a dipeptidyl peptidase 8 polypeptide is needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multi-functional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene). In a BLUESCRIPT vector, a sequence encoding the dipeptidyl peptidase 8 polypeptide can be ligated into the vector in frame with sequences for the amino-terminal 10 Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem.* 264, 5503-5509, 1989) or pGEX vectors (Promega, Madison, Wis.) also can be used to express 15 foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free 20 glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

25 In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH can be used. For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol.* 153, 516-544, 1987.

Plant and Insect Expression Systems

If plant expression vectors are used, the expression of sequences encoding dipeptidyl peptidase 8 polypeptides can be driven by any of a number of promoters. For 5 example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV (Takamatsu, *EMBO J.* 6, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J.* 3, 1671-1680, 1984; Broglie *et al.*, *Science* 224, 838-843, 1984; Winter *et al.*, *Results 10 Probl. Cell Differ.* 17, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (e.g., Hobbs or Murray, in *MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY*, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

15

An insect system also can be used to express a dipeptidyl peptidase 8 polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding dipeptidyl peptidase 8 polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of 20 dipeptidyl peptidase 8 polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which dipeptidyl peptidase 8 polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci.* 91, 25 3224-3227, 1994).

Mammalian Expression Systems

30 A number of viral-based expression systems can be used to express dipeptidyl peptidase 8 polypeptides in mammalian host cells. For example, if an adenovirus is

used as an expression vector, sequences encoding dipeptidyl peptidase 8 polypeptides can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus that is capable of

5 expressing a dipeptidyl peptidase 8 polypeptide in infected host cells (Logan & Shenk, *Proc. Natl. Acad. Sci.* 81, 3655-3659, 1984). If desired, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

10 Human artificial chromosomes (HACs) also can be used to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (e.g., liposomes, polycationic amino polymers, or vesicles).

15 Specific initiation signals also can be used to achieve more efficient translation of sequences encoding dipeptidyl peptidase 8 polypeptides. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding a dipeptidyl peptidase 8 polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins,

20 both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf *et al.*, *Results Probl. Cell Differ.* 20, 125-162, 1994).

25

Host Cells

A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed dipeptidyl peptidase 8 polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells that have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express dipeptidyl peptidase 8 polypeptides can be transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced dipeptidyl peptidase 8 sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type. See, for example, ANIMAL CELL CULTURE, R.I. Freshney, ed., 1986.

Any number of selection systems can be used to recover transformed cell lines.

These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell* 11, 223-32, 1977) and adenine phosphoribosyltransferase (Lowy

et al., Cell 22, 817-23, 1980) genes which can be employed in *tk*⁻ or *aprt*⁻ cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al., Proc. Natl. Acad. Sci. 77, 3567-70, 1980*), *npt* confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin *et al., J. Mol. Biol. 150, 1-14, 1981*), and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murray, 1992, *supra*). Additional selectable genes have been described. For example, *trpB* allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. 85, 8047-51, 1988*). Visible markers such as anthocyanins, β -glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al., Methods Mol. Biol. 55, 121-131, 1995*).

15

Detecting Expression

Although the presence of marker gene expression suggests that the dipeptidyl peptidase 8 polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding a dipeptidyl peptidase 8 polypeptide is inserted within a marker gene sequence, transformed cells containing sequences that encode a dipeptidyl peptidase 8 polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a dipeptidyl peptidase 8 polypeptide under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the dipeptidyl peptidase 8 polynucleotide.

30 Alternatively, host cells which contain a dipeptidyl peptidase 8 polynucleotide and which express a dipeptidyl peptidase 8 polypeptide can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or

immunoassay techniques that include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein. For example, the presence of a polynucleotide sequence encoding a dipeptidyl peptidase 8 polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or 5 amplification using probes or fragments or fragments of polynucleotides encoding a dipeptidyl peptidase 8 polypeptide. Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding a dipeptidyl peptidase 8 polypeptide to detect transformants that contain a dipeptidyl peptidase 8 polynucleotide.

10

A variety of protocols for detecting and measuring the expression of a dipeptidyl peptidase 8 polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include enzyme-linked immuno-sorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a dipeptidyl peptidase 8 polypeptide can be used, or a competitive binding assay can be employed. These and other assays are described in Hampton *et al.*, SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990 and Maddox *et al.*, *J. Exp. Med.* 158, 1211-1216, 1983).

20 A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to 25 polynucleotides encoding dipeptidyl peptidase 8 polypeptides include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding a dipeptidyl peptidase 8 polypeptide can be cloned 30 into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially

available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

5

Expression and Purification of Polypeptides

Host cells transformed with nucleotide sequences encoding a dipeptidyl peptidase 8 polypeptide can be cultured under conditions suitable for the expression and recovery 10 of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing 15 polynucleotides which encode dipeptidyl peptidase 8 polypeptides can be designed to contain signal sequences which direct secretion of soluble dipeptidyl peptidase 8 polypeptides through a prokaryotic or eukaryotic cell membrane or which direct the membrane insertion of membrane-bound dipeptidyl peptidase 8 polypeptide.

As discussed above, other constructions can be used to join a sequence encoding a dipeptidyl peptidase 8 polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification 20 facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., 25 Seattle, Wash.). Inclusion of cleavable linker sequences such as those specific for Factor Xa or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the dipeptidyl peptidase 8 polypeptide also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a dipeptidyl peptidase 8 polypeptide and 6 histidine residues preceding a 30 thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilized metal ion affinity chromatography, as described

in Porath *et al.*, *Prot. Exp. Purif.* 3, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying the dipeptidyl peptidase 8 polypeptide from the fusion protein. Vectors that contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol.* 12, 441-453, 1993.

5

Chemical Synthesis

Sequences encoding a dipeptidyl peptidase 8 polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, 10 *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al.* *Nucl. Acids Res. Symp. Ser.* 225-232, 1980). Alternatively, a dipeptidyl peptidase 8 polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc.* 85, 2149-2154, 1963; Roberge *et al.*, *Science* 269, 202-204, 1995). Protein synthesis 15 can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of dipeptidyl peptidase 8 polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

20

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (e.g., Creighton, *PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES*, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic dipeptidyl peptidase 8 polypeptide can be confirmed by 25 amino acid analysis or sequencing (e.g., the Edman degradation procedure; *see* Creighton, *supra*). Additionally, any portion of the amino acid sequence of the dipeptidyl peptidase 8 polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

30

Production of Altered Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce
5 dipeptidyl peptidase 8 polypeptide-encoding nucleotide sequences possessing
non-naturally occurring codons. For example, codons preferred by a particular
prokaryotic or eukaryotic host can be selected to increase the rate of protein
expression or to produce an RNA transcript having desirable properties, such as a
half-life that is longer than that of a transcript generated from the naturally occurring
sequence.

10

The nucleotide sequences disclosed herein can be engineered using methods
generally known in the art to alter dipeptidyl peptidase 8 polypeptide-encoding
sequences for a variety of reasons, including but not limited to, alterations which
modify the cloning, processing, and/or expression of the polypeptide or mRNA
15 product. DNA shuffling by random fragmentation and PCR reassembly of gene
fragments and synthetic oligonucleotides can be used to engineer the nucleotide
sequences. For example, site-directed mutagenesis can be used to insert new
restriction sites, alter glycosylation patterns, change codon preference, produce splice
variants, introduce mutations, and so forth.

20

Antibodies

Any type of antibody known in the art can be generated to bind specifically to an
epitope of a dipeptidyl peptidase 8 polypeptide. "Antibody" as used herein includes
25 intact immunoglobulin molecules, as well as fragments thereof, such as Fab, F(ab')₂,
and Fv, which are capable of binding an epitope of a dipeptidyl peptidase 8
polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to
form an epitope. However, epitopes which involve non-contiguous amino acids may
require more, e.g., at least 15, 25, or 50 amino acids.

30

An antibody which specifically binds to an epitope of a dipeptidyl peptidase 8 polypeptide can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various 5 immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody that specifically binds to the immunogen.

10 Typically, an antibody which specifically binds to a dipeptidyl peptidase 8 polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to dipeptidyl peptidase 8 15 polypeptides do not detect other proteins in immunochemical assays and can immunoprecipitate a dipeptidyl peptidase 8 polypeptide from solution.

20 Human dipeptidyl peptidase 8 polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, a dipeptidyl peptidase 8 polypeptide can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet 25 hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (e.g., aluminum hydroxide), and surface active substances (e.g. lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

30 Monoclonal antibodies that specifically bind to a dipeptidyl peptidase 8 polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not

limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler *et al.*, *Nature* 256, 495-497, 1985; Kozbor *et al.*, *J. Immunol. Methods* 81, 31-42, 1985; Cote *et al.*, *Proc. Natl. Acad. Sci.* 80, 2026-2030, 1983; Cole *et al.*, *Mol. Cell Biol.* 62, 109-120, 1984).

5

In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, *Proc. Natl. Acad. Sci.* 81, 6851-6855, 1984; Neuberger *et al.*, *Nature* 312, 604-608, 1984; Takeda *et al.*, *Nature* 314, 452-454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grafting of entire complementarity determining regions. Alternatively, humanized antibodies can be produced using recombinant methods, as described in GB2188638B. Antibodies that specifically bind to a dipeptidyl peptidase 8 polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies that specifically bind to dipeptidyl peptidase 8 polypeptides. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobulin libraries (Burton, *Proc. Natl. Acad. Sci.* 88, 11120-23, 1991).

30 Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J.*

- 30 -

5 *Cancer Prev.* 5, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol.* 15, 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem.* 269, 199-206.

10 A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*, 1995, *Int. J. Cancer* 61, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth.* 165, 81-91).

15 Antibodies which specifically bind to dipeptidyl peptidase 8 polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci.* 86, 3833-3837, 1989; Winter *et al.*, *Nature* 349, 293-299, 1991).

20 Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

25 Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which a dipeptidyl peptidase 8 polypeptide is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences that are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary 5 nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a 10 cell as described above to decrease the level of dipeptidyl peptidase 8 gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an automated 15 synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such alkyl-phosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamide, carboxymethyl esters, carbonates, and phosphate triesters. See Brown, *Meth. Mol. Biol.* 20, 1-8, 1994; Sonveaux, *Meth. Mol. Biol.* 26, 1-72, 1994; Uhlmann *et al.*, 20 *Chem. Rev.* 90, 543-583, 1990.

Modifications of dipeptidyl peptidase 8 gene expression can be obtained by designing 25 antisense oligonucleotides that will form duplexes to the control, 5', or regulatory regions of the dipeptidyl peptidase 8 gene. Oligonucleotides derived from the transcription initiation site, *e.g.*, between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability 30 of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee *et al.*, in Huber & Carr, MOLECULAR AND IMMUNOLOGIC

APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

5 Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of a dipeptidyl peptidase 8 polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 10 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a dipeptidyl peptidase 8 polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent dipeptidyl peptidase 8 nucleotides, can provide sufficient targeting specificity for dipeptidyl peptidase 8 mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 15 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular dipeptidyl peptidase 8 polynucleotide sequence.

20 Antisense oligonucleotides can be modified without affecting their ability to hybridize to a dipeptidyl peptidase 8 polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or 25 a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. *See, e.g., Agrawal et al., Trends Biotechnol. 10, 152-158, 1992; Uhlmann et al., Chem. Rev. 90, 543-584, 1990; Uhlmann et al., Tetrahedron. Lett. 30 215, 3539-3542, 1987.*

Ribozymes

Ribozymes are RNA molecules with catalytic activity. *See, e.g., Cech, Science 236, 1532-1539; 1987; Cech, Ann. Rev. Biochem. 59, 543-568; 1990, Cech, Curr. Opin. Struct. Biol. 2, 605-609; 1992, Couture & Stinchcomb, Trends Genet. 12, 510-515, 1996.* Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (e.g., Haseloff *et al.*, U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

The coding sequence of a dipeptidyl peptidase 8 polynucleotide can be used to generate ribozymes that will specifically bind to mRNA transcribed from the dipeptidyl peptidase 8 polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (*see Haseloff *et al.* Nature 334, 585-591, 1988*). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.*, EP 321,201).

Specific ribozyme cleavage sites within a dipeptidyl peptidase 8 RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate dipeptidyl peptidase 8 RNA targets also can be evaluated by testing accessibility to hybridization.

zation with complementary oligonucleotides using ribonuclease protection assays. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the 5 ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical 10 methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease dipeptidyl peptidase 8 expression. Alternatively, if it is desired that the cells stably retain the DNA 15 construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so 20 that ribozyme expression will occur in response to factors that induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

25 **Differentially Expressed Genes**

Described herein are methods for the identification of genes whose products interact 30 with human dipeptidyl peptidase 8. Such genes may represent genes that are differentially expressed in disorders including, but not limited to, cancer, CNS disorders, and COPD. Further, such genes may represent genes that are differentially regulated in response to manipulations relevant to the progression or treatment of

such diseases. Additionally, such genes may have a temporally modulated expression, increased or decreased at different stages of tissue or organism development. A differentially expressed gene may also have its expression modulated under control versus experimental conditions. In addition, the human dipeptidyl peptidase 5 gene or gene product may itself be tested for differential expression.

10 The degree to which expression differs in a normal versus a diseased state need only be large enough to be visualized via standard characterization techniques such as differential display techniques. Other such standard characterization techniques by which expression differences may be visualized include but are not limited to, quantitative RT (reverse transcriptase), PCR, and Northern analysis.

Identification of Differentially Expressed Genes

15 To identify differentially expressed genes total RNA or, preferably, mRNA is isolated from tissues of interest. For example, RNA samples are obtained from tissues of experimental subjects and from corresponding tissues of control subjects. Any RNA isolation technique that does not select against the isolation of mRNA may be utilized for the purification of such RNA samples. See, for example, Ausubel *et al.*, ed., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, Inc. New York, 1987-1993. Large numbers of tissue samples may readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski, U.S. Patent 4,843,155.

20

25 Transcripts within the collected RNA samples that represent RNA produced by differentially expressed genes are identified by methods well known to those of skill in the art. They include, for example, differential screening (Tedder *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 85, 208-12, 1988), subtractive hybridization (Hedrick *et al.*, *Nature* 308, 149-53; Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88, 2825, 1984), and,

30 preferably, differential display (Liang & Pardee, *Science* 257, 967-71, 1992; U.S. Patent 5,262,311).

The differential expression information may itself suggest relevant methods for the treatment of disorders involving the human dipeptidyl peptidase 8. For example, treatment may include a modulation of expression of the differentially expressed 5 genes and/or the gene encoding the human dipeptidyl peptidase 8. The differential expression information may indicate whether the expression or activity of the differentially expressed gene or gene product or the human dipeptidyl peptidase 8 gene or gene product are up-regulated or down-regulated.

10 Screening Methods

The invention provides assays for screening test compounds that bind to or modulate the activity of a dipeptidyl peptidase 8 polypeptide or a dipeptidyl peptidase 8 polynucleotide. A test compound preferably binds to a dipeptidyl peptidase 8 polypeptide 15 or polynucleotide. More preferably, a test compound decreases or increases dipeptidyl peptidase activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

20 Test Compounds

Test compounds can be pharmacologic agents already known in the art or can be 25 compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and 30 synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches

are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. *See* Lam, *Anticancer Drug Des.* 12, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (see, for 5 example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 90, 6909, 1993; Erb *et al.* *Proc. Natl. Acad. Sci. U.S.A.* 91, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem.* 37, 2678, 1994; Cho *et al.*, *Science* 261, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.* 33, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.* 33, 2061; Gallop *et al.*, *J. Med. Chem.* 37, 1233, 1994). Libraries of compounds can be presented in solution 10 (see, e.g., Houghten, *BioTechniques* 13, 412-421, 1992), or on beads (Lam, *Nature* 354, 82-84, 1991), chips (Fodor, *Nature* 364, 555-556, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 89, 1865-1869, 1992), or phage (Scott & Smith, *Science* 249, 386-390, 1990; Devlin, *Science* 249, 404-406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci.* 97, 6378-6382, 15 1990; Felici, *J. Mol. Biol.* 222, 301-310, 1991; and Ladner, U.S. Patent 5,223,409).

High Throughput Screening

Test compounds can be screened for the ability to bind to dipeptidyl peptidase 8 20 polypeptides or polynucleotides or to affect dipeptidyl peptidase 8 activity or dipeptidyl peptidase 8 gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates 25 typically require assay volumes that range from 50 to 500 μ l. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, "free format assays," or assays that have no physical barrier between 30 samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by

5 Jayawickreme *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 91, 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

10 Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the 15 compounds were partially released by UV-light. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

20 Yet another example is described by Salmon *et al.*, *Molecular Diversity* 2, 57-63 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

25 Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

Binding Assays

For binding assays, the test compound is preferably a small molecule that binds to and occupies, for example, the active site of the dipeptidyl peptidase 8 polypeptide, 5 such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules.

In binding assays, either the test compound or the dipeptidyl peptidase 8 polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, 10 or luciferase. Detection of a test compound that is bound to the dipeptidyl peptidase 8 polypeptide can then be accomplished, for example, by direct counting of radio-emission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product.

15 Alternatively, binding of a test compound to a dipeptidyl peptidase 8 polypeptide can be determined without labeling either of the interactants. For example, a microphysiometer can be used to detect binding of a test compound with a dipeptidyl peptidase 8 polypeptide. A microphysiometer (e.g., CytosensorTM) is an analytical 20 instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and a dipeptidyl peptidase 8 polypeptide (McConnell *et al.*, *Science* 257, 1906-1912, 1992).

25 Determining the ability of a test compound to bind to a dipeptidyl peptidase 8 polypeptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem.* 63, 2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol.* 5, 699-705, 1995). BIA is a 30 technology for studying biospecific interactions in real time, without labeling any of the interactants (e.g., BIACoreTM). Changes in the optical phenomenon surface

plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In yet another aspect of the invention, a dipeptidyl peptidase 8 polypeptide can be
5 used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent 5,283,317; Zervos *et al.*, *Cell* 72, 223-232, 1993; Madura *et al.*, *J. Biol. Chem.* 268, 12046-12054, 1993; Bartel *et al.*, *BioTechniques* 14, 920-924, 1993; Iwabuchi *et al.*, *Oncogene* 8, 1693-1696, 1993; and Brent W094/10300), to identify other proteins which bind to or interact with the dipeptidyl peptidase 8 polypeptide and
10 modulate its activity.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct, polynucleotide
15 encoding a dipeptidyl peptidase 8 polypeptide can be fused to a polynucleotide encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct a DNA sequence that encodes an unidentified protein ("prey" or "sample") can be fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo* to form an protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the
20 functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein that interacts with the dipeptidyl peptidase 8 polypeptide.

It may be desirable to immobilize either the dipeptidyl peptidase 8 polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the dipeptidyl peptidase 8 polypeptide (or polynucleotide) or the
30

test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the

5 enzyme polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide (or polynucleotide) or test compound and the solid support. Test compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked.

10 Binding of a test compound to a dipeptidyl peptidase 8 polypeptide (or polynucleotide) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

15 In one embodiment, the dipeptidyl peptidase 8 polypeptide is a fusion protein comprising a domain that allows the dipeptidyl peptidase 8 polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test

20 compound or the test compound and the non-adsorbed dipeptidyl peptidase 8 polypeptide; the mixture is then incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as

25 described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either a dipeptidyl peptidase 8 polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated dipeptidyl peptidase 8

30

polypeptides (or polynucleotides) or test compounds can be prepared from biotin-NHS(N-hydroxysuccinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which

5 specifically bind to a dipeptidyl peptidase 8 polypeptide, polynucleotide, or a test compound, but which do not interfere with a desired binding site, such as the active site of the dipeptidyl peptidase 8 polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

10 Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to the dipeptidyl peptidase 8 polypeptide or test compound, enzyme-linked assays which rely on detecting an activity of the dipeptidyl peptidase 8 polypeptide, and SDS gel electrophoresis under non-reducing conditions.

15 Screening for test compounds which bind to a dipeptidyl peptidase 8 polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises a dipeptidyl peptidase 8 polypeptide or polynucleotide can be used in a cell-based assay system. A dipeptidyl peptidase 8 polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above.

20 Binding of the test compound to a dipeptidyl peptidase 8 polypeptide or polynucleotide is determined as described above.

Enzyme Assays

25 Test compounds can be tested for the ability to increase or decrease the dipeptidyl peptidase activity of a human dipeptidyl peptidase 8 polypeptide. Dipeptidyl peptidase activity can be measured, for example, as described in Maes *et al.*, *Neuro-psychopharmacology*. 2001 Feb;24(2):130-40; Sentandreu & Toldra, *J Agric Food Chem.* 2000 Oct;48(10):5014-22; Li *et al.*, *Biochem Biophys Res Commun.* 2000 Sep 24;276(2):553-8; or Durinx *et al.*, *Eur J Biochem.* 2000 Sep;267(17):5608-13.

Enzyme assays can be carried out after contacting either a purified dipeptidyl peptidase 8 polypeptide, a cell membrane preparation, or an intact cell with a test compound. A test compound that decreases a dipeptidyl peptidase activity of a dipeptidyl peptidase 8 polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential therapeutic agent for decreasing dipeptidyl peptidase 8 activity. A test compound which increases a dipeptidyl peptidase activity of a human dipeptidyl peptidase 8 polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential therapeutic agent for increasing human dipeptidyl peptidase 8 activity.

Gene Expression

In another embodiment, test compounds that increase or decrease dipeptidyl peptidase 8 gene expression are identified. A dipeptidyl peptidase 8 polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the dipeptidyl peptidase 8 polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

The level of dipeptidyl peptidase 8 mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence of polypeptide

products of a dipeptidyl peptidase 8 polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into a dipeptidyl peptidase 8 polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell that expresses a dipeptidyl peptidase 8 polynucleotide can be used in a cell-based assay system. The dipeptidyl peptidase 8 polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, such as CHO or human embryonic kidney 293 cells, can be used.

15 Pharmaceutical Compositions

The invention also provides pharmaceutical compositions that can be administered to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise, for example, a dipeptidyl peptidase 8 polypeptide, dipeptidyl peptidase 8 polynucleotide, ribozymes or antisense oligonucleotides, antibodies which specifically bind to a dipeptidyl peptidase 8 polypeptide, or mimetics, activators, or inhibitors of a dipeptidyl peptidase 8 polypeptide activity. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

30 In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries that facilitate processing of the active compounds into preparations which can be

used pharmaceutically. Pharmaceutical compositions of the invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

10

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

25

Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

30

Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a

filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

5

Pharmaceutical formulations suitable for parenteral administration can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances that increase the viscosity of the suspension, such 10 as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the 15 suspension also can contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

20

The pharmaceutical compositions of the present invention can be manufactured in a manner that is known in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt 25 and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% 30 mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

- 47 -

Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa.). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

5

Therapeutic Indications and Methods

Human dipeptidyl peptidase 8 can be regulated to treat cancer, CNS disorders, and COPD.

10

Cancer

Cancer is a disease fundamentally caused by oncogenic cellular transformation. There are several hallmarks of transformed cells that distinguish them from their normal counterparts and underlie the pathophysiology of cancer. These include uncontrolled cellular proliferation, unresponsiveness to normal death-inducing signals (immortalization), increased cellular motility and invasiveness, increased ability to recruit blood supply through induction of new blood vessel formation (angiogenesis), genetic instability, and dysregulated gene expression. Various combinations of these aberrant physiologies, along with the acquisition of drug-resistance frequently lead to an intractable disease state in which organ failure and patient death ultimately ensue.

15

Most standard cancer therapies target cellular proliferation and rely on the differential proliferative capacities between transformed and normal cells for their efficacy. This approach is hindered by the facts that several important normal cell types are also highly proliferative and that cancer cells frequently become resistant to these agents. Thus, the therapeutic indices for traditional anti-cancer therapies rarely exceed 2.0.

20

25

30

The advent of genomics-driven molecular target identification has opened up the possibility of identifying new cancer-specific targets for therapeutic intervention that will provide safer, more effective treatments for cancer patients. Thus, newly discovered tumor-associated genes and their products can be tested for their role(s) in 5 disease and used as tools to discover and develop innovative therapies. Genes playing important roles in any of the physiological processes outlined above can be characterized as cancer targets.

10 Genes or gene fragments identified through genomics can readily be expressed in one or more heterologous expression systems to produce functional recombinant proteins. These proteins are characterized *in vitro* for their biochemical properties and then used as tools in high-throughput molecular screening programs to identify chemical modulators of their biochemical activities. Activators and/or inhibitors of target 15 protein activity can be identified in this manner and subsequently tested in cellular and *in vivo* disease models for anti-cancer activity. Optimization of lead compounds with iterative testing in biological models and detailed pharmacokinetic and toxicological analyses form the basis for drug development and subsequent testing in humans.

20 CNS disorders

Central and peripheral nervous system disorders also can be treated, such as primary 25 and secondary disorders after brain injury, disorders of mood, anxiety disorders, disorders of thought and volition, disorders of sleep and wakefulness, diseases of the motor unit, such as neurogenic and myopathic disorders, neurodegenerative disorders such as Alzheimer's and Parkinson's disease, and processes of peripheral and chronic pain.

30 Pain that is associated with CNS disorders also can be treated by regulating the activity of human dipeptidyl peptidase 8. Pain which can be treated includes that associated with central nervous system disorders, such as multiple sclerosis, spinal

cord injury, sciatica, failed back surgery syndrome, traumatic brain injury, epilepsy, Parkinson's disease, post-stroke, and vascular lesions in the brain and spinal cord (e.g., infarct, hemorrhage, vascular malformation). Non-central neuropathic pain includes that associated with post mastectomy pain, reflex sympathetic dystrophy (RSD), trigeminal neuralgia/oculopathy, post-surgical pain, HIV/AIDS related pain, cancer pain, metabolic neuropathies (e.g., diabetic neuropathy, vasculitic neuropathy secondary to connective tissue disease), paraneoplastic polyneuropathy associated, for example, with carcinoma of lung, or leukemia, or lymphoma, or carcinoma of prostate, colon or stomach, trigeminal neuralgia, cranial neuralgias, and post-herpetic neuralgia. Pain associated with cancer and cancer treatment also can be treated, as can headache pain (for example, migraine with aura, migraine without aura, and other migraine disorders), episodic and chronic tension-type headache, tension-type like headache, cluster headache, and chronic paroxysmal hemicrania.

15 COPD

Chronic obstructive pulmonary (or airways) disease (COPD) is a condition defined physiologically as airflow obstruction that generally results from a mixture of emphysema and peripheral airway obstruction due to chronic bronchitis (Senior & 20 Shapiro, *Pulmonary Diseases and Disorders*, 3d ed., New York, McGraw-Hill, 1998, pp. 659-681, 1998; Barnes, *Chest* 117, 10S-14S, 2000). Emphysema is characterized by destruction of alveolar walls leading to abnormal enlargement of the air spaces of the lung. Chronic bronchitis is defined clinically as the presence of chronic productive cough for three months in each of two successive years. In COPD, airflow 25 obstruction is usually progressive and is only partially reversible. By far the most important risk factor for development of COPD is cigarette smoking, although the disease does occur in non-smokers.

Chronic inflammation of the airways is a key pathological feature of COPD (Senior 30 & Shapiro, 1998). The inflammatory cell population comprises increased numbers of macrophages, neutrophils, and CD8⁺ lymphocytes. Inhaled irritants, such as cigarette

smoke, activate macrophages which are resident in the respiratory tract, as well as epithelial cells leading to release of chemokines (e.g., interleukin-8) and other chemotactic factors. These chemotactic factors act to increase the neutrophil-/monocyte trafficking from the blood into the lung tissue and airways. Neutrophils 5 and monocytes recruited into the airways can release a variety of potentially damaging mediators such as proteolytic enzymes and reactive oxygen species. Matrix degradation and emphysema, along with airway wall thickening, surfactant dysfunction, and mucus hypersecretion, all are potential sequelae of this inflammatory response that lead to impaired airflow and gas exchange.

10

This invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For example, an agent identified as described herein (e.g., a modulating agent, an 15 antisense nucleic acid molecule, a specific antibody, ribozyme, or a dipeptidyl peptidase 8 polypeptide binding molecule) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model 20 to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

A reagent which affects dipeptidyl peptidase 8 activity can be administered to a 25 human cell, either *in vitro* or *in vivo*, to reduce dipeptidyl peptidase 8 activity. The reagent preferably binds to an expression product of a human dipeptidyl peptidase 8 gene. If the expression product is a protein, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a preparation of stem cells that have been removed from the body. The cells can then be replaced in 30 the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of targeting to a specific organ of an animal, such as the lung, liver, spleen, heart brain, lymph nodes, and skin.

10 A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μ g of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μ g of DNA per 16 nmole of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μ g of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

15 20 Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a particular cell type, such as a cell-specific ligand exposed on the outer surface of the liposome.

25 30 Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods that are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 μ g to about 10 μ g of polynucleotide is combined with about 8 nmol of liposomes, more preferably from

about 0.5 μ g to about 5 μ g of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 μ g of polynucleotides is combined with about 8 nmol liposomes.

5 In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al.* *Trends in Biotechnol.* 11, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem.* 263, 621-24 (1988);
10 Wu *et al.*, *J. Biol. Chem.* 269, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 87, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem.* 266, 338-42 (1991).

Determination of a Therapeutically Effective Dose

15 The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases dipeptidyl peptidase 8 activity relative to the dipeptidyl peptidase 8 activity which occurs in the absence of the therapeutically effective dose.
20 For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.
25

30 Therapeutic efficacy and toxicity, *e.g.*, ED₅₀ (the dose therapeutically effective in 50% of the population) and LD₅₀ (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD₅₀/ED₅₀.

5 Pharmaceutical compositions that exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

10 The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors that can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and 15 tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

20 Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations 25 for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

30 If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads,

protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

Effective *in vivo* dosages of an antibody are in the range of about 5 μ g to about 5 50 μ g/kg, about 50 μ g to about 5 mg/kg, about 100 μ g to about 500 μ g/kg of patient body weight, and about 200 to about 250 μ g/kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg, about 1 μ g to about 2 mg, about 5 μ g to about 500 μ g, and about 20 μ g to about 10 100 μ g of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides that express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

15 Preferably, a reagent reduces expression of a dipeptidyl peptidase 8 gene or the activity of a dipeptidyl peptidase 8 polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of a 20 dipeptidyl peptidase 8 gene or the activity of a dipeptidyl peptidase 8 polypeptide can be assessed using methods well known in the art, such as hybridization of nucleotide probes to dipeptidyl peptidase 8-specific mRNA, quantitative RT-PCR, immunologic detection of a dipeptidyl peptidase 8 polypeptide, or measurement of dipeptidyl peptidase 8 activity.

25 In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical 30 principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described above. Using this

approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

5 Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Diagnostic Methods

10 Human dipeptidyl peptidase 8 also can be used in diagnostic assays for detecting diseases and abnormalities or susceptibility to diseases and abnormalities related to the presence of mutations in the nucleic acid sequences that encode the enzyme. For example, differences can be determined between the cDNA or genomic sequence encoding dipeptidyl peptidase 8 in individuals afflicted with a disease and in normal individuals. If a mutation is observed in some or all of the afflicted individuals but not in normal individuals, then the mutation is likely to be the causative agent of the disease.

20 Sequence differences between a reference gene and a gene having mutations can be revealed by the direct DNA sequencing method. In addition, cloned DNA segments can be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer can be used with a double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by 25 conventional procedures using radiolabeled nucleotides or by automatic sequencing procedures using fluorescent tags.

30 Genetic testing based on DNA sequence differences can be carried out by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized, for example, by high resolution gel electrophoresis. DNA fragments of different se-

- 56 -

quences can be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers *et al.*, *Science* 230, 1242, 1985). Sequence changes at specific locations can also be 5 revealed by nuclease protection assays, such as RNase and S 1 protection or the chemical cleavage method (e.g., Cotton *et al.*, *Proc. Natl. Acad. Sci. USA* 85, 4397-4401, 1985). Thus, the detection of a specific DNA sequence can be performed by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes and Southern blotting of genomic DNA. 10 In addition to direct methods such as gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

Altered levels of dipeptidyl peptidase 8 also can be detected in various tissues. Assays used to detect levels of the receptor polypeptides in a body sample, such as 15 blood or a tissue biopsy, derived from a host are well known to those of skill in the art and include radioimmunoassays, competitive binding assays, Western blot analysis, and ELISA assays.

All patents and patent applications cited in this disclosure are expressly incorporated 20 herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples, which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1*Detection of dipeptidyl peptidase 8 activity*

5 The polynucleotide of SEQ ID NO: 1 is inserted into the expression vector pCEV4 and the expression vector pCEV4-dipeptidyl peptidase 8 polypeptide obtained is transfected into human embryonic kidney 293 cells. From these cells extracts are obtained and incubated with substrate in 70 µl phosphate buffer, pH 7.4, for 30 min at 37°C. The specific DPPIV substrates are Gly-Pro-toluenesulfonate, H-Gly-Pro-p-
10 nitroanilide (NA)/HCl (Sigma, St. Louis, MO, USA) and Gly-Pro-7-amino-4-trifluoromethylcoumarin (Calbiochem, San Diego, CA, USA). It is shown that the polypeptide of SEQ ID NO: 2 hydrolyzes the DPPIV substrates. Thus this polypeptide has a dipeptidyl peptidase activity.

15 **EXAMPLE 2***Expression of recombinant human dipeptidyl peptidase 8*

20 The *Pichia pastoris* expression vector pPICZB (Invitrogen, San Diego, CA) is used to produce large quantities of recombinant human dipeptidyl peptidase 8 polypeptides in yeast. The dipeptidyl peptidase 8-encoding DNA sequence is derived from SEQ ID NO: 1. Before insertion into vector pPICZB, the DNA sequence is modified by well known methods in such a way that it contains at its 5'-end an initiation codon and at its 3'-end an enterokinase cleavage site, a His6 reporter tag
25 and a termination codon. Moreover, at both termini recognition sequences for restriction endonucleases are added and after digestion of the multiple cloning site of pPICZB with the corresponding restriction enzymes the modified DNA sequence is ligated into pPICZB. This expression vector is designed for inducible expression in *Pichia pastoris*, driven by a yeast promoter. The resulting pPICZB/nd-His6 vector is
30 used to transform the yeast.

The yeast is cultivated under usual conditions in 5 liter shake flasks and the recombinantly produced protein isolated from the culture by affinity chromatography (Ni-NTA-Resin) in the presence of 8 M urea. The bound polypeptide is eluted with buffer, pH 3.5, and neutralized. Separation of the polypeptide from the His6 reporter tag is accomplished by site-specific proteolysis using enterokinase (Invitrogen, San Diego, CA) according to manufacturer's instructions. Purified human dipeptidyl peptidase 8 polypeptide is obtained.

EXAMPLE 3

10

Identification of test compounds that bind to dipeptidyl peptidase 8 polypeptides

Purified dipeptidyl peptidase 8 polypeptides comprising a glutathione-S-transferase protein and absorbed onto glutathione-derivatized wells of 96-well microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. Human dipeptidyl peptidase 8 polypeptides comprise the amino acid sequence shown in SEQ ID NO: 2. The test compounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

20

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to a dipeptidyl peptidase 8 polypeptide is detected by fluorescence measurements of the contents of the wells. A test compound that increases the fluorescence in a well by at least 15% relative to fluorescence of a well in which a test compound is not incubated is identified as a compound which binds to a dipeptidyl peptidase 8 polypeptide.

EXAMPLE 4

Identification of a test compound which decreases dipeptidyl peptidase 8 gene expression

5

A test compound is administered to a culture of human cells transfected with a dipeptidyl peptidase 8 expression construct and incubated at 37°C for 10 to 45 minutes. A culture of the same type of cells that have not been transfected is incubated for the same time without the test compound to provide a negative control.

10

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem.* 18, 5294-99, 1979). Northern blots are prepared using 20 to 30 µg total RNA and hybridized with a ³²P-labeled dipeptidyl peptidase 8-specific probe at 65°C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from the complement of SEQ ID NO: 1. A test compound that decreases the dipeptidyl peptidase 8-specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of dipeptidyl peptidase 8 gene expression.

15

20

EXAMPLE 5

Identification of a test compound which decreases dipeptidyl peptidase 8 activity

25

A test compound is administered to a culture of human cells transfected with a dipeptidyl peptidase 8 expression construct and incubated at 37°C for 10 to 45 minutes. A culture of the same type of cells that have not been transfected is incubated for the same time without the test compound to provide a negative control. Dipeptidyl peptidase activity is measured using the method of Maes *et al.*, *Neuropsychopharmacology*. 2001 Feb;24(2):130-40; Sentandreu & Toldra, *J Agric Food Chem.* 2000 Oct;48(10):5014-22; Li *et al.*, *Biochem Biophys Res Commun.*

30

2000 Sep 24;276(2):553-8; or Durinx *et al.*, Eur J Biochem. 2000 Sep;267(17):5608-13.

5 A test compound which decreases the dipeptidyl peptidase activity of the dipeptidyl peptidase 8 relative to the dipeptidyl peptidase activity in the absence of the test compound is identified as an inhibitor of dipeptidyl peptidase 8 activity.

EXAMPLE 6

10 *Tissue-specific expression of dipeptidyl peptidase 8*

The qualitative expression pattern of dipeptidyl peptidase 8 in various tissues is determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR).

15 To demonstrate that dipeptidyl peptidase 8 is involved in the disease process of COPD, the initial expression panel consists of RNA samples from respiratory tissues and inflammatory cells relevant to COPD: lung (adult and fetal), trachea, freshly isolated alveolar type II cells, cultured human bronchial epithelial cells, cultured small airway epithelial cells, cultured bronchial smooth muscle cells, cultured H441 cells (Clara-like), freshly isolated neutrophils and monocytes, and cultured monocytes (macrophage-like). Body map profiling also is carried out, using total RNA panels purchased from Clontech. The tissues are adrenal gland, bone marrow, brain, colon, heart, kidney, liver, lung, mammary gland, pancreas, prostate, salivary gland, skeletal muscle, small intestine, spleen, stomach, testis, thymus, trachea, thyroid, and uterus.

20

25

30 To demonstrate that dipeptidyl peptidase 8 is involved in CNS disorders, the following tissues are screened: fetal and adult brain, muscle, heart, lung, kidney, liver, thymus, testis, colon, placenta, trachea, pancreas, kidney, gastric mucosa, colon, liver, cerebellum, skin, cortex (Alzheimer's and normal), hypothalamus,

cortex, amygdala, cerebellum, hippocampus, choroid, plexus, thalamus, and spinal cord.

To demonstrate that dipeptidyl peptidase 8 is involved in cancer, expression is
5 determined in the following tissues: adrenal gland, bone marrow, brain, cerebellum, colon, fetal brain, fetal liver, heart, kidney, liver, lung, mammary gland, pancreas, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thymus, thyroid, trachea, uterus, and peripheral blood lymphocytes. Expression in the following cancer cell lines also is determined: DU-145 (prostate),
10 NCI-H125 (lung), HT-29 (colon), COLO-205 (colon), A-549 (lung), NCI-H460 (lung), HT-116 (colon), DLD-1 (colon), MDA-MD-231 (breast), LS174T (colon), ZF-75 (breast), MDA-MN-435 (breast), HT-1080, MCF-7 (breast), and U87. Matched pairs of malignant and normal tissue from the same patient also are tested.

15 *Quantitative expression profiling.* Quantitative expression profiling is performed by the form of quantitative PCR analysis called "kinetic analysis" firstly described in Higuchi *et al.*, *BioTechnology* 10, 413-17, 1992, and Higuchi *et al.*, *BioTechnology* 11, 1026-30, 1993. The principle is that at any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template
20 copies.

If the amplification is performed in the presence of an internally quenched
25 fluorescent oligonucleotide (TaqMan probe) complementary to the target sequence, the probe is cleaved by the 5'-3' endonuclease activity of Taq DNA polymerase and a fluorescent dye released in the medium (Holland *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88, 7276-80, 1991). Because the fluorescence emission will increase in direct proportion to the amount of the specific amplified product, the exponential growth phase of PCR product can be detected and used to determine the initial template concentration (Heid *et al.*, *Genome Res.* 6, 986-94, 1996, and Gibson *et al.*, *Genome Res.* 6, 995-1001, 1996).

- 62 -

The amplification of an endogenous control can be performed to standardize the amount of sample RNA added to a reaction. In this kind of experiment, the control of choice is the 18S ribosomal RNA. Because reporter dyes with differing emission spectra are available, the target and the endogenous control can be independently quantified in the same tube if probes labeled with different dyes are used.

5

All "real time PCR" measurements of fluorescence are made in the ABI Prism 7700.

10

RNA extraction and cDNA preparation. Total RNA from the tissues listed above are used for expression quantification. RNAs labeled "from autopsy" were extracted from autoptic tissues with the TRIzol reagent (Life Technologies, MD) according to the manufacturer's protocol.

15

Fifty µg of each RNA were treated with DNase I for 1 hour at 37°C in the following reaction mix: 0.2 U/µl RNase-free DNase I (Roche Diagnostics, Germany); 0.4 U/µl RNase inhibitor (PE Applied Biosystems, CA); 10 mM Tris-HCl pH 7.9; 10 mM MgCl₂; 50 mM NaCl; and 1 mM DTT.

20

After incubation, RNA is extracted once with 1 volume of phenol:chloroform:isoamyl alcohol (24:24:1) and once with chloroform, and precipitated with 1/10 volume of 3 M NaAcetate, pH5.2, and 2 volumes of ethanol.

25

Fifty µg of each RNA from the autoptic tissues are DNase treated with the DNA-free kit purchased from Ambion (Ambion, TX). After resuspension and spectrophotometric quantification, each sample is reverse transcribed with the TaqMan Reverse Transcription Reagents (PE Applied Biosystems, CA) according to the manufacturer's protocol. The final concentration of RNA in the reaction mix is 200 ng/µL. Reverse transcription is carried out with 2.5µM of random hexamer primers.

30

5 *TaqMan quantitative analysis.* Specific primers and probe are designed according to the recommendations of PE Applied Biosystems; the probe can be labeled at the 5' end FAM (6-carboxy-fluorescein) and at the 3' end with TAMRA (6-carboxy-tetramethyl-rhodamine). Quantification experiments are performed on 10 ng of reverse transcribed RNA from each sample. Each determination is done in triplicate.

10 Total cDNA content is normalized with the simultaneous quantification (multiplex PCR) of the 18S ribosomal RNA using the Pre-Developed TaqMan Assay Reagents (PDAR) Control Kit (PE Applied Biosystems, CA).

15 The assay reaction mix is as follows: 1X final TaqMan Universal PCR Master Mix (from 2X stock) (PE Applied Biosystems, CA); 1X PDAR control – 18S RNA (from 20X stock); 300 nM forward primer; 900 nM reverse primer; 200 nM probe; 10 ng cDNA; and water to 25 μ l.

20 Each of the following steps are carried out once: pre PCR, 2 minutes at 50°C, and 10 minutes at 95°C. The following steps are carried out 40 times: denaturation, 15 seconds at 95°C, annealing/extension, 1 minute at 60°C.

25 The experiment is performed on an ABI Prism 7700 Sequence Detector (PE Applied Biosystems, CA). At the end of the run, fluorescence data acquired during PCR are processed as described in the ABI Prism 7700 user's manual in order to achieve better background subtraction as well as signal linearity with the starting target quantity.

30

EXAMPLE 7

Proliferation inhibition assay: Antisense oligonucleotides suppress the growth of cancer cell lines

30

The cell line used for testing is the human colon cancer cell line HCT116. Cells are cultured in RPMI-1640 with 10-15% fetal calf serum at a concentration of 10,000

cells per milliliter in a volume of 0.5 ml and kept at 37°C in a 95% air/5%CO₂ atmosphere.

5 Phosphorothioate oligoribonucleotides are synthesized on an Applied Biosystems Model 380B DNA synthesizer using phosphoroamidite chemistry. A sequence of 24 bases complementary to the nucleotides at position 1 to 24 of SEQ ID NO: 1 is used as the test oligonucleotide. As a control, another (random) sequence is used: 5'-TCA ACT GAC TAG ATG TAC ATG GAC-3'. Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate buffered saline at the desired concentration. Purity of the oligonucleotides is tested by capillary gel electrophoresis and ion exchange HPLC. The purified oligonucleotides are added to the culture medium at a concentration of 10 µM once per day for seven days.

10 15 The addition of the test oligonucleotide for seven days results in significantly reduced expression of human dipeptidyl peptidase 8 as determined by Western blotting. This effect is not observed with the control oligonucleotide. After 3 to 7 days, the number of cells in the cultures is counted using an automatic cell counter. The number of cells in cultures treated with the test oligonucleotide (expressed as 100%) is compared with the number of cells in cultures treated with the control oligonucleotide. The number of cells in cultures treated with the test oligonucleotide is not more than 30% of control, indicating that the inhibition of human dipeptidyl peptidase 8 has an anti-proliferative effect on cancer cells.

20 25 **EXAMPLE 8**

In vivo testing of compounds/target validation

30 1. **Acute Mechanistic Assays**
 1.1. ***Reduction in Mitogenic Plasma Hormone Levels***

This non-tumor assay measures the ability of a compound to reduce either the endogenous level of a circulating hormone or the level of hormone produced in

response to a biologic stimulus. Rodents are administered test compound (p.o., i.p., i.v., i.m., or s.c.). At a predetermined time after administration of test compound, blood plasma is collected. Plasma is assayed for levels of the hormone of interest. If the normal circulating levels of the hormone are too low and/or variable to provide 5 consistent results, the level of the hormone may be elevated by a pre-treatment with a biologic stimulus (i.e., LHRH may be injected i.m. into mice at a dosage of 30 ng/mouse to induce a burst of testosterone synthesis). The timing of plasma collection would be adjusted to coincide with the peak of the induced hormone response. Compound effects are compared to a vehicle-treated control group. An F- 10 test is preformed to determine if the variance is equal or unequal followed by a Student's t-test. Significance is p value \leq 0.05 compared to the vehicle control group.

1.2. Hollow Fiber Mechanism of Action Assay

15

Hollow fibers are prepared with desired cell line(s) and implanted intraperitoneally and/or subcutaneously in rodents. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Fibers are harvested in accordance with specific readout assay protocol, these 20 may include assays for gene expression (bDNA, PCR, or Taqman), or a specific biochemical activity (i.e., cAMP levels. Results are analyzed by Student's t-test or Rank Sum test after the variance between groups is compared by an F-test, with significance at p \leq 0.05 as compared to the vehicle control group.

2. Subacute Functional *In Vivo* Assays

25

2.1. Reduction in Mass of Hormone Dependent Tissues

This is another non-tumor assay that measures the ability of a compound to reduce the mass of a hormone dependent tissue (i.e., seminal vesicles in males and uteri in 30 females). Rodents are administered test compound (p.o., i.p., i.v., i.m., or s.c.) according to a predetermined schedule and for a predetermined duration (i.e., 1

week). At termination of the study, animals are weighed, the target organ is excised, any fluid is expressed, and the weight of the organ is recorded. Blood plasma may also be collected. Plasma may be assayed for levels of a hormone of interest or for levels of test agent. Organ weights may be directly compared or they may be 5 normalized for the body weight of the animal. Compound effects are compared to a vehicle-treated control group. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test. Significance is p value ≤ 0.05 compared to the vehicle control group.

10 **2.2. *Hollow Fiber Proliferation Assay***

Hollow fibers are prepared with desired cell line(s) and implanted intraperitoneally and/or subcutaneously in rodents. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Fibers are harvested in accordance with specific readout assay protocol. Cell 15 proliferation is determined by measuring a marker of cell number (i.e., MTT or LDH). The cell number and change in cell number from the starting inoculum are analyzed by Student's t-test or Rank Sum test after the variance between groups is compared by an F-test, with significance at $p \leq 0.05$ as compared to the vehicle control group.

20

2.3. *Anti-angiogenesis Models*

2.3.1. *Corneal Angiogenesis*

25 Hydron pellets with or without growth factors or cells are implanted into a micro-pocket surgically created in the rodent cornea. Compound administration may be systemic or local (compound mixed with growth factors in the hydron pellet). Corneas are harvested at 7 days post implantation immediately following intracardiac infusion of colloidal carbon and are fixed in 10% formalin. Readout is qualitative scoring and/or image analysis. Qualitative scores are compared by Rank Sum test. 30 Image analysis data is evaluated by measuring the area of neovascularization (in

pixels) and group averages are compared by Student's t-test (2 tail). Significance is $p \leq 0.05$ as compared to the growth factor or cells only group.

2.3.2. *Matrigel Angiogenesis*

5

Matrigel, containing cells or growth factors, is injected subcutaneously. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Matrigel plugs are harvested at pre-determined time point(s) and prepared for readout. Readout is an ELISA-based assay for hemoglobin concentration and/or histological examination (i.e. vessel count, 10 special staining for endothelial surface markers: CD31, factor-8). Readouts are analyzed by Student's t-test, after the variance between groups is compared by an F-test, with significance determined at $p \leq 0.05$ as compared to the vehicle control group.

15

3. Primary Antitumor Efficacy

3.1. *Early Therapy Models*

20

3.1.1. *Subcutaneous Tumor*

Tumor cells or fragments are implanted subcutaneously on Day 0. Vehicle and/or compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule starting at a time, usually on Day 1, prior to the ability to measure the tumor burden. Body weights and tumor measurements are recorded 2-3 times weekly. 25 Mean net body and tumor weights are calculated for each data collection day. Antitumor efficacy may be initially determined by comparing the size of treated (T) and control (C) tumors on a given day by a Student's t-test, after the variance between groups is compared by an F-test, with significance determined at $p \leq 0.05$. The experiment may also be continued past the end of dosing in which case tumor measurements would continue to be recorded to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and 30

control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is $p \leq 0.05$.

5

3.1.2. Intraperitoneal/Intracranial Tumor Models

Tumor cells are injected intraperitoneally or intracranially on Day 0. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule 10 starting on Day 1. Observations of morbidity and/or mortality are recorded twice daily. Body weights are measured and recorded twice weekly. Morbidity/mortality data is expressed in terms of the median time of survival and the number of long-term survivors is indicated separately. Survival times are used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control 15 group in the experiment.

3.2. Established Disease Model

Tumor cells or fragments are implanted subcutaneously and grown to the desired size 20 for treatment to begin. Once at the predetermined size range, mice are randomized into treatment groups. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Tumor and body weights are measured and recorded 2-3 times weekly. Mean tumor weights of all groups over days post inoculation are graphed for comparison. An F-test is preformed to determine if the 25 variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. Tumor measurements may be recorded after dosing has stopped to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a 30 predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by generating Kaplan-Meier curves from the times

for individual tumors to attain the evaluation size. Significance is $p \text{ value} \leq 0.05$ compared to the vehicle control group.

3.3. *Orthotopic Disease Models*

5

3.3.1. *Mammary Fat Pad Assay*

Tumor cells or fragments, of mammary adenocarcinoma origin, are implanted directly into a surgically exposed and reflected mammary fat pad in rodents. The fat pad is placed back in its original position and the surgical site is closed. Hormones may also be administered to the rodents to support the growth of the tumors. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Tumor and body weights are measured and recorded 2-3 times weekly. Mean tumor weights of all groups over days post inoculation are graphed for comparison. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group.

10

15

20

25

30

Tumor measurements may be recorded after dosing has stopped to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is $p \text{ value} \leq 0.05$ compared to the vehicle control group. In addition, this model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ, or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

3.3.2. *Intraprostatic Assay*

Tumor cells or fragments, of prostatic adenocarcinoma origin, are implanted directly
5 into a surgically exposed dorsal lobe of the prostate in rodents. The prostate is externalized through an abdominal incision so that the tumor can be implanted specifically in the dorsal lobe while verifying that the implant does not enter the seminal vesicles. The successfully inoculated prostate is replaced in the abdomen and the incisions through the abdomen and skin are closed. Hormones may also be
10 administered to the rodents to support the growth of the tumors. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer
15 attached to a dissecting scope. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at
20 termination of the study by counting the number of visible foci per target organ (i.e., the lungs), or measuring the target organ weight (i.e., the regional lymph nodes). The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

25

3.3.3. *Intrabronchial Assay*

Tumor cells of pulmonary origin may be implanted intrabronchially by making an incision through the skin and exposing the trachea. The trachea is pierced with the
30 beveled end of a 25 gauge needle and the tumor cells are inoculated into the main bronchus using a flat-ended 27 gauge needle with a 90° bend. Compounds are

administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at 5 termination of the study by counting the number of visible foci per target organ (i.e., the contralateral lung), or measuring the target organ weight. The means of these 10 endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

15 3.3.4. *Intracecal Assay.*

Tumor cells of gastrointestinal origin may be implanted intracecally by making an abdominal incision through the skin and externalizing the intestine. Tumor cells are inoculated into the cecal wall without penetrating the lumen of the intestine using a 20 27 or 30 gauge needle. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is 25 preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at 30 termination of the study by counting the number of visible foci per target organ (i.e., the liver), or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test,

with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

4. Secondary (Metastatic) Antitumor Efficacy

5

4.1. *Spontaneous Metastasis*

Tumor cells are inoculated s.c. and the tumors allowed to grow to a predetermined range for spontaneous metastasis studies to the lung or liver. These primary tumors are then excised. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule which may include the period leading up to the excision of the primary tumor to evaluate therapies directed at inhibiting the early stages of tumor metastasis. Observations of morbidity and/or mortality are recorded daily. Body weights are measured and recorded twice weekly. Potential endpoints include 10 survival time, numbers of visible foci per target organ, or target organ weight. When survival time is used as the endpoint the other values are not determined. Survival data is used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment. The mean number of visible tumor foci, as determined under a dissecting microscope, and the mean target organ 15 weights are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment for both of these endpoints.

15

4.2. *Forced Metastasis*

25

Tumor cells are injected into the tail vein, portal vein, or the left ventricle of the heart in experimental (forced) lung, liver, and bone metastasis studies, respectively. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Observations of morbidity and/or mortality are recorded daily. Body 20 weights are measured and recorded twice weekly. Potential endpoints include survival time, numbers of visible foci per target organ, or target organ weight. When

- 73 -

survival time is used as the endpoint the other values are not determined. Survival data is used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment. The mean number of visible tumor foci, as determined under a dissecting microscope, and the mean target organ 5 weights are compared by Student's t-test after conducting an F-test, with significance at $p \leq 0.05$ compared to the vehicle control group in the experiment for both endpoints.

EXAMPLE 9

10

In vivo testing of compounds/target validation

1. Pain

15

Acute Pain

20

Acute pain is measured on a hot plate mainly in rats. Two variants of hot plate testing are used: In the classical variant animals are put on a hot surface (52 to 56°C) and the latency time is measured until the animals show nocifensive behavior, such as stepping or foot licking. The other variant is an increasing temperature hot plate where the experimental animals are put on a surface of neutral temperature. Subsequently this surface is slowly but constantly heated until the animals begin to lick a hind paw. The temperature which is reached when hind paw licking begins is a measure for pain threshold.

25

Compounds are tested against a vehicle treated control group. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

30

Persistent Pain

Persistent pain is measured with the formalin or capsaicin test, mainly in rats.

5 A solution of 1 to 5% formalin or 10 to 100 µg capsaicin is injected into one hind paw of the experimental animal. After formalin or capsaicin application the animals show nocifensive reactions like flinching, licking and biting of the affected paw. The number of nocifensive reactions within a time frame of up to 90 minutes is a measure for intensity of pain.

10 Compounds are tested against a vehicle treated control group. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to formalin or capsaicin administration.

15 Neuropathic Pain

Neuropathic pain is induced by different variants of unilateral sciatic nerve injury mainly in rats. The operation is performed under anesthesia. The first variant of sciatic nerve injury is produced by placing loosely constrictive ligatures around the common sciatic nerve. The second variant is the tight ligation of about the half of the diameter of the common sciatic nerve. In the next variant, a group of models is used in which tight ligations or transections are made of either the L5 and L6 spinal nerves, or the L7 spinal nerve only. The fourth variant involves an axotomy of two of the three terminal branches of the sciatic nerve (tibial and common peroneal nerves) leaving the remaining sural nerve intact whereas the last variant comprises the axotomy of only the tibial branch leaving the sural and common nerves uninjured. Control animals are treated with a sham operation.

30 Postoperatively, the nerve injured animals develop a chronic mechanical allodynia, cold allodynia, as well as a thermal hyperalgesia. Mechanical

allodynia is measured by means of a pressure transducer (electronic von Frey Anesthesiometer, IITC Inc.-Life Science Instruments, Woodland Hills, SA, USA; Electronic von Frey System, Somedic Sales AB, Hörby, Sweden). Thermal hyperalgesia is measured by means of a radiant heat source (Plantar Test, Ugo Basile, Comerio, Italy), or by means of a cold plate of 5 to 10°C where the nocifensive reactions of the affected hind paw are counted as a measure of pain intensity. A further test for cold induced pain is the counting of nocifensive reactions, or duration of nocifensive responses after plantar administration of acetone to the affected hind limb. Chronic pain in general is assessed by registering the circadian rhythms in activity (Surjo and Arndt, Universität zu Köln, Cologne, Germany), and by scoring differences in gait (foot print patterns; FOOTPRINTS program, Klapdor et al., 1997. A low cost method to analyze footprint patterns. *J. Neurosci. Methods* 75, 49-54).

15 Compounds are tested against sham operated and vehicle treated control groups. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

20 ***Inflammatory Pain***

25 Inflammatory pain is induced mainly in rats by injection of 0.75 mg carrageenan or complete Freund's adjuvant into one hind paw. The animals develop an edema with mechanical allodynia as well as thermal hyperalgesia. Mechanical allodynia is measured by means of a pressure transducer (electronic von Frey Anesthesiometer, IITC Inc.-Life Science Instruments, Woodland Hills, SA, USA). Thermal hyperalgesia is measured by means of a radiant heat source (Plantar Test, Ugo Basile, Comerio, Italy, Paw thermal stimulator, G. Ozaki, University of California, USA). For edema measurement two methods are being used. In the first method, the animals are sacrificed and the affected hindpaws sectioned and weighed. The second

- 76 -

method comprises differences in paw volume by measuring water displacement in a plethysmometer (Ugo Basile, Comerio, Italy).

5 Compounds are tested against uninflamed as well as vehicle treated control groups. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

Diabetic Neuropathic Pain

10 Rats treated with a single intraperitoneal injection of 50 to 80 mg/kg streptozotocin develop a profound hyperglycemia and mechanical allodynia within 1 to 3 weeks. Mechanical allodynia is measured by means of a pressure transducer (electronic von Frey Anesthesiometer, IITC Inc.-Life Science Instruments, Woodland Hills, CA, USA).

15 20 Compounds are tested against diabetic and non-diabetic vehicle treated control groups. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

2. Parkinson's disease

6-Hydroxydopamine (6-OH-DA) Lesion

25 Degeneration of the dopaminergic nigrostriatal and striatopallidal pathways is the central pathological event in Parkinson's disease. This disorder has been mimicked experimentally in rats using single/sequential unilateral stereotaxic injections of 6-OH-DA into the medium forebrain bundle (MFB).

Male Wistar rats (Harlan Winkelmann, Germany), weighing 200±250 g at the beginning of the experiment, are used. The rats are maintained in a temperature- and humidity-controlled environment under a 12 h light/dark cycle with free access to food and water when not in experimental sessions.

5 The following in vivo protocols are approved by the governmental authorities. All efforts are made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques.

10 Animals are administered pargyline on the day of surgery (Sigma, St. Louis, MO, USA; 50 mg/kg i.p.) in order to inhibit metabolism of 6-OHDA by monoamine oxidase and desmethylimipramine HCl (Sigma; 25 mg/kg i.p.) in order to prevent uptake of 6-OHDA by noradrenergic terminals. Thirty minutes later the rats are anesthetized with sodium pentobarbital (50 mg/kg) and placed in a stereotaxic frame. In order to lesion the DA nigrostriatal 15 pathway 4 µl of 0.01% ascorbic acid-saline containing 8 µg of 6-OHDA HBr (Sigma) are injected into the left medial fore-brain bundle at a rate of 1 µl/min (2.4 mm anterior, 1.49 mm lateral, -2.7 mm ventral to Bregma and the skull surface). The needle is left in place an additional 5 min to allow diffusion to occur.

20

Stepping Test

25 Forelimb akinesia is assessed three weeks following lesion placement using a modified stepping test protocol. In brief, the animals are held by the experimenter with one hand fixing the hindlimbs and slightly raising the hind part above the surface. One paw is touching the table, and is then moved slowly sideways (5 s for 1 m), first in the forehand and then in the backhand direction. The number of adjusting steps is counted for both paws in the backhand and forehand direction of movement. The sequence of testing is 30 right paw forehand and backhand adjusting stepping, followed by left paw forehand and backhand directions. The test is repeated three times on three

consecutive days, after an initial training period of three days prior to the first testing. Forehand adjusted stepping reveals no consistent differences between lesioned and healthy control animals. Analysis is therefore restricted to backhand adjusted stepping.

5

Balance Test

Balance adjustments following postural challenge are also measured during the stepping test sessions. The rats are held in the same position as described in the stepping test and, instead of being moved sideways, tilted by the experimenter towards the side of the paw touching the table. This maneuver results in loss of balance and the ability of the rats to regain balance by forelimb movements is scored on a scale ranging from 0 to 3. Score 0 is given for a normal forelimb placement. When the forelimb movement is delayed but recovery of postural balance detected, score 1 is given. Score 2 represents a clear, yet insufficient, forelimb reaction, as evidenced by muscle contraction, but lack of success in recovering balance, and score 3 is given for no reaction of movement. The test is repeated three times a day on each side for three consecutive days after an initial training period of three days prior to the first testing.

10

15

20

Staircase Test (Paw Reaching)

25

30

A modified version of the staircase test is used for evaluation of paw reaching behavior three weeks following primary and secondary lesion placement. Plexiglass test boxes with a central platform and a removable staircase on each side are used. The apparatus is designed such that only the paw on the same side at each staircase can be used, thus providing a measure of independent forelimb use. For each test the animals are left in the test boxes for 15 min. The double staircase is filled with 7 x 3 chow pellets (Precision food pellets, formula: P, purified rodent diet, size 45 mg; Sandown Scientific)

- 79 -

on each side. After each test the number of pellets eaten (successfully retrieved pellets) and the number of pellets taken (touched but dropped) for each paw and the success rate (pellets eaten/pellets taken) are counted separately. After three days of food deprivation (12 g per animal per day) the 5 animals are tested for 11 days. Full analysis is conducted only for the last five days.

MPTP treatment

10 The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP) causes degeneration of mesencephalic dopaminergic (DAergic) neurons in rodents, non-human primates, and humans and, in so doing, reproduces many of the symptoms of Parkinson's disease. MPTP leads to a marked decrease in the levels of dopamine and its metabolites, and in the number of dopaminergic terminals in the striatum as well as severe loss of the tyrosine hydroxylase (TH)-immunoreactive cell bodies in the substantia nigra, pars compacta. 15

20 In order to obtain severe and long-lasting lesions, and to reduce mortality, animals receive single injections of MPTP, and are then tested for severity of lesion 7–10 days later. Successive MPTP injections are administered on days 1, 2 and 3. Animals receive application of 4 mg/kg MPTP hydrochloride (Sigma) in saline once daily. All injections are intraperitoneal (i.p.) and the MPTP stock solution is frozen between injections. Animals are decapitated on day 11. 25

Immunohistology

30 At the completion of behavioral experiments, all animals are anaesthetized with 3 ml thiopental (1 g/40 ml i.p., Tyrol Pharma). The mice are perfused transcardially with 0.01 M PBS (pH 7.4) for 2 min, followed by 4% paraformaldehyde (Merck) in PBS for 15 min. The brains are removed and placed in

- 80 -

4% paraformaldehyde for 24 h at 4°C. For dehydration they are then transferred to a 20% sucrose (Merck) solution in 0.1 M PBS at 4°C until they sink. The brains are frozen in methylbutan at -20°C for 2 min and stored at -70°C. Using a sledge microtome (mod. 3800-Frigocut, Leica), 25 µm sections are 5 taken from the genu of the corpus callosum (AP 1.7 mm) to the hippocampus (AP 21.8 mm) and from AP 24.16 to AP 26.72. Forty-six sections are cut and stored in assorters in 0.25 M Tris buffer (pH 7.4) for immunohistochemistry.

10 A series of sections is processed for free-floating tyrosine hydroxylase (TH) immunohistochemistry. Following three rinses in 0.1 M PBS, endogenous peroxidase activity is quenched for 10 min in 0.3% H₂O₂ ± PBS. After rinsing in PBS, sections are preincubated in 10% normal bovine serum (Sigma) for 5 min as blocking agent and transferred to either primary anti-rat TH rabbit antiserum (dilution 1:2000).

15 Following overnight incubation at room temperature, sections for TH immunoreactivity are rinsed in PBS (2 x10 min) and incubated in biotinylated anti-rabbit immunoglobulin G raised in goat (dilution 1:200) (Vector) for 90 min, rinsed repeatedly and transferred to Vectastain ABC (Vector) solution 20 for 1 h. 3,3' -Diaminobenzidine tetrahydrochloride (DAB; Sigma) in 0.1 M PBS, supplemented with 0.005% H₂O₂, serves as chromogen in the subsequent visualization reaction. Sections are mounted on to gelatin-coated slides, left to dry overnight, counter-stained with hematoxylin dehydrated in ascending alcohol concentrations and cleared in butylacetate. Coverslips are 25 mounted on entellan.

Rotarod Test

30 We use a modification of the procedure described by Rozas and Labandeira-Garcia (1997), with a CR-1 Rotarnex system (Columbus Instruments, Columbus, OH) comprising an IBM-compatible personal computer, a CIO-24

data acquisition card, a control unit, and a four-lane rotarod unit. The rotarod unit consists of a rotating spindle (diameter 7.3 cm) and individual compartments for each mouse. The system software allows preprogramming of session protocols with varying rotational speeds (0–80 rpm). Infrared beams are used to detect when a mouse has fallen onto the base grid beneath the rotarod. The system logs the fall as the end of the experiment for that mouse, and the total time on the rotarod, as well as the time of the fall and all the set-up parameters, are recorded. The system also allows a weak current to be passed through the base grid, to aid training.

10

3. **Dementia**

The object recognition task

15 The object recognition task has been designed to assess the effects of experimental manipulations on the cognitive performance of rodents. A rat is placed in an open field, in which two identical objects are present. The rats inspects both objects during the first trial of the object recognition task. In a second trial, after a retention interval of for example 24 hours, one of the two objects used in the first trial, the 'familiar' object, and a novel object are placed in the open field. The inspection time at each of the objects is registered. The basic measures in the OR task is the time spent by a rat exploring the two object the second trial. Good retention is reflected by higher exploration times towards the novel than the 'familiar' object.

20

25 Administration of the putative cognition enhancer prior to the first trial predominantly allows assessment of the effects on acquisition, and eventually on consolidation processes. Administration of the testing compound after the first trial allows to assess the effects on consolidation processes, whereas administration before the second trial allows to measure effects on retrieval processes.

30

The passive avoidance task

The passive avoidance task assesses memory performance in rats and mice.

5 The inhibitory avoidance apparatus consists of a two-compartment box with a light compartment and a dark compartment. The two compartments are separated by a guillotine door that can be operated by the experimenter. A threshold of 2 cm separates the two compartments when the guillotine door is raised. When the door is open, the illumination in the dark compartment is about 2 lux. The light intensity is about 500 lux at the center of the floor of

10 the light compartment.

15 Two habituation sessions, one shock session, and a retention session are given, separated by inter-session intervals of 24 hours. In the habituation sessions and the retention session the rat is allowed to explore the apparatus for 300 sec. The rat is placed in the light compartment, facing the wall opposite to the guillotine door. After an accommodation period of 15 sec. the guillotine door is opened so that all parts of the apparatus can be visited freely. Rats normally avoid brightly lit areas and will enter the dark

20 compartment within a few seconds.

25 In the shock session the guillotine door between the compartments is lowered as soon as the rat has entered the dark compartment with its four paws, and a scrambled 1 mA footshock is administered for 2 sec. The rat is removed from the apparatus and put back into its home cage. The procedure during the retention session is identical to that of the habituation sessions.

30 The step-through latency, that is the first latency of entering the dark compartment (in sec.) during the retention session is an index of the memory performance of the animal; the longer the latency to enter the dark compartment, the better the retention is. A testing compound is given half an

- 83 -

5

hour before the shock session, together with 1 mg*kg⁻¹ scopolamine. Scopolamine impairs the memory performance during the retention session 24 hours later. If the test compound increases the enter latency compared with the scopolamine-treated controls, is likely to possess cognition enhancing potential.

10

The Morris water escape task
The Morris water escape task measures spatial orientation learning in rodents. It is a test system that has extensively been used to investigate the effects of putative therapeutic on the cognitive functions of rats and mice. The performance of an animal is assessed in a circular water tank with an escape platform that is submerged about 1 cm below the surface of the water. The escape platform is not visible for an animal swimming in the water tank. Abundant extra-maze cues are provided by the furniture in the room, including desks, computer equipment, a second water tank, the presence of the experimenter, and by a radio on a shelf that is playing softly.

15

20

The animals receive four trials during five daily acquisition sessions. A trial is started by placing an animal into the pool, facing the wall of the tank. Each of four starting positions in the quadrants north, east, south, and west is used once in a series of four trials; their order is randomized. The escape platform is always in the same position. A trial is terminated as soon as the animal had climbs onto the escape platform or when 90 seconds have elapsed, whichever event occurs first. The animal is allowed to stay on the platform for 30 seconds. Then it is taken from the platform and the next trial is started. If an animal did not find the platform within 90 seconds it is put on the platform by the experimenter and is allowed to stay there for 30 seconds. After the fourth trial of the fifth daily session, an additional trial is given as a probe trial: the platform is removed, and the time the animal spends in the four quadrants is measured for 30 or 60 seconds. In the probe trial, all animals start from the

25

30

same start position, opposite to the quadrant where the escape platform had been positioned during acquisition.

5 Four different measures are taken to evaluate the performance of an animal during acquisition training: escape latency, traveled distance, distance to platform, and swimming speed. The following measures are evaluated for the probe trial: time (s) in quadrants and traveled distance (cm) in the four quadrants. The probe trial provides additional information about how well an animal learned the position of the escape platform. If an animal spends more 10 time and swims a longer distance in the quadrant where the platform had been positioned during the acquisition sessions than in any other quadrant, one concludes that the platform position has been learned well.

15 In order to assess the effects of putative cognition enhancing compounds, rats or mice with specific brain lesions which impair cognitive functions, or animals treated with compounds such as scopolamine or MK-801, which interfere with normal learning, or aged animals which suffer from cognitive deficits, are used.

20 *The T-maze spontaneous alternation task*

25 The T-maze spontaneous alternation task (TeMCAT) assesses the spatial memory performance in mice. The start arm and the two goal arms of the T-maze are provided with guillotine doors which can be operated manually by the experimenter. A mouse is put into the start arm at the beginning of training. The guillotine door is closed. In the first trial, the 'forced trial', either the left or right goal arm is blocked by lowering the guillotine door. After the mouse has been released from the start arm, it will negotiate the maze, eventually enter the open goal arm, and return to the start position, 30 where it will be confined for 5 seconds, by lowering the guillotine door. Then, the animal can choose freely between the left and right goal arm (all

guillotine-doors opened) during 14 'free choice' trials. As soon a the mouse has entered one goal arm, the other one is closed. The mouse eventually returns to the start arm and is free to visit whichever go alarm it wants after having been confined to the start arm for 5 seconds. After completion of 14 free choice trials in one session, the animal is removed from the maze. During 5 training, the animal is never handled.

The percent alternations out of 14 trials is calculated. This percentage and the total time needed to complete the first forced trial and the subsequent 14 free 10 choice trials (in s) is analyzed. Cognitive deficits are usually induced by an injection of scopolamine, 30 min before the start of the training session. Scopolamine reduced the per-cent alternations to chance level, or below. A cognition enhancer, which is always administered before the training session, will at least partially, antagonize the scopolamine-induced reduction in the 15 spontaneous alternation rate.

REFERENCES

1. Abbott CA, Yu DM, Woollatt E, Sutherland GR, McCaughan GW, Gorrell 20 MD. Cloning, expression and chromosomal localization of a novel human dipeptidyl peptidase (DPP) IV homolog, DPP8. *Eur J Biochem* 2000 Oct;267(20):6140-50
2. Wilson MJ, Ruhland AR, Quast BJ, Reddy PK, Ewing SL, Sinha AA. 25 Dipeptidylpeptidase IV activities are elevated in prostate cancers and adjacent benign hyperplastic glands. *J Androl* 2000 Mar-Apr;21(2):220-6
3. Raikhlin NT, Bukaeva IA, Probatova NA, Smirnova EA, Pavlovskaya AI, Tupitsyn NN, Sholokhova EN. Dipeptidyl aminopeptidase-IV as a 30 histochemical marker of differential diagnosis of large cell anaplastic CD30+-lymphoma and Hodgkin's disease. *Arkh Patol* 2000 Jul-Aug;62(4):3-8

- 86 -

4. Hildebrandt M, Reutter W, Arck P, Rose M, Klapp BF. A guardian angel: the involvement of dipeptidyl peptidase IV in psychoneuroendocrine function, nutrition and immune defense. *Clin Sci (Colch)* 2000 Aug 1;99(2):93-104
5. Umeki K, Yamamoto I, Maruta J, Aratake Y, Ono I, Uemura Y, Tanaka T, Toyoda K, Kotani T, Noguchi S, Sakamoto F, Konoe K, Ohtaki S. CD26/dipeptidyl peptidase IV and thyroid peroxidase as molecular markers for differentiated thyroid carcinoma. *Rinsho Byori* 1996 Jan;44(1):42-50
10. Van Der Velden VH, Naber BA, Van Hal PT, Overbeek SE, Hoogsteden HC, Versnel MA. Peptidase activities in serum and bronchoalveolar lavage fluid from allergic asthmatics--comparison with healthy non-smokers and smokers and effects of inhaled glucocorticoids. *Clin Exp Allergy* 1999 Jun;29(6):813-15
15. 23

CLAIMS

1. An isolated polynucleotide being selected from the group consisting of:
 - 5 a) a polynucleotide encoding a dipeptidyl peptidase 8 polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 62% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.
 - 10 b) a polynucleotide comprising the sequence of SEQ ID NO: 1;
 - c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b) and encodes a dipeptidyl peptidase 8 polypeptide;
 - 15 d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code and encodes a dipeptidyl peptidase 8 polypeptide; and
 - 20 e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d) and encodes a dipeptidyl peptidase 8 polypeptide.
- 25 2. An expression vector containing any polynucleotide of claim 1.
3. A host cell containing the expression vector of claim 2.
- 30 4. A substantially purified dipeptidyl peptidase 8 polypeptide encoded by a polynucleotide of claim 1.

5. A method for producing a dipeptidyl peptidase 8 polypeptide, wherein the method comprises the following steps:
 - 5 a) culturing the host cell of claim 3 under conditions suitable for the expression of the dipeptidyl peptidase 8 polypeptide; and
 - 10 b) recovering the dipeptidyl peptidase 8 polypeptide from the host cell culture.
- 15 6. A method for detection of a polynucleotide encoding a dipeptidyl peptidase 8 polypeptide in a biological sample comprising the following steps:
 - a) hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and
 - 15 b) detecting said hybridization complex.
- 20 7. The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.
- 25 8. A method for the detection of a polynucleotide of claim 1 or a dipeptidyl peptidase 8 polypeptide of claim 4 comprising the steps of:
 - contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the dipeptidyl peptidase 8 polypeptide.
9. A diagnostic kit for conducting the method of any one of claims 6 to 8.
- 30 10. A method of screening for agents which decrease the activity of a dipeptidyl peptidase 8, comprising the steps of:

contacting a test compound with any dipeptidyl peptidase 8 polypeptide encoded by any polynucleotide of claim 1;

5 detecting binding of the test compound to the dipeptidyl peptidase 8 polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential therapeutic agent for decreasing the activity of a dipeptidyl peptidase 8.

10 11. A method of screening for agents which regulate the activity of a dipeptidyl
peptidase 8, comprising the steps of:

contacting a test compound with a dipeptidyl peptidase 8 polypeptide encoded by any polynucleotide of claim 1; and

15 detecting a dipeptidyl peptidase 8 activity of the polypeptide, wherein a test compound which increases the dipeptidyl peptidase 8 activity is identified as a potential therapeutic agent for increasing the activity of the dipeptidyl peptidase 8, and wherein a test compound which decreases the dipeptidyl peptidase 8 activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of the dipeptidyl peptidase 8.
20

12. A method of screening for agents which decrease the activity of a dipeptidyl peptidase 8, comprising the steps of:

25

- contacting a test compound with any polynucleotide of claim 1 and detecting binding of the test compound to the polynucleotide, wherein a test compound which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of dipeptidyl peptidase 8.

30

13. A method of reducing the activity of dipeptidyl peptidase 8, comprising the steps of:
 - 5 contacting a cell with a reagent which specifically binds to any polynucleotide of claim 1 or any dipeptidyl peptidase 8 polypeptide of claim 4, whereby the activity of dipeptidyl peptidase 8 is reduced.
14. A reagent that modulates the activity of a dipeptidyl peptidase 8 polypeptide or a polynucleotide wherein said reagent is identified by the method of any of
 - 10 the claim 10 to 12.
15. A pharmaceutical composition, comprising:
 - 15 the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.
16. Use of the expression vector of claim 2 or the reagent of claim 14 in the preparation of a medicament for modulating the activity of a dipeptidyl peptidase 8 in a disease.
 - 20
 17. Use of claim 16 wherein the disease is, cancer, a CNS disorder or COPD.
 18. A cDNA encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2.
 - 25
 19. The cDNA of claim 18 which comprises SEQ ID NO: 1.
 20. The cDNA of claim 18 which consists of SEQ ID NO: 1.
 - 30
 21. An expression vector comprising a polynucleotide which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2.

22. The expression vector of claim 21 wherein the polynucleotide consists of SEQ ID NO: 1.
- 5 23. A host cell comprising an expression vector which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2.
24. The host cell of claim 23 wherein the polynucleotide consists of SEQ ID NO: 1.
- 10 25. A purified polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2.
26. The purified polypeptide of claim 25 which consists of the amino acid sequence shown in SEQ ID NO: 2.
- 15 27. A fusion protein comprising a polypeptide having the amino acid sequence shown in SEQ ID NO: 2.
- 20 28. A method of producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, comprising the steps of:
 - culturing a host cell comprising an expression vector which encodes the polypeptide under conditions whereby the polypeptide is expressed; and
 - 25 isolating the polypeptide.
29. The method of claim 28 wherein the expression vector comprises SEQ ID NO: 1.

30. A method of detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, comprising the steps of:
 - 5 hybridizing a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO: 1 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and
 - 10 detecting the hybridization complex.
- 15 31. The method of claim 30 further comprising the step of amplifying the nucleic acid material before the step of hybridizing.
32. A kit for detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, comprising:
 - 15 a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO: 1; and instructions for the method of claim 30.
 - 20 33. A method of detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, comprising the steps of:
 - 25 contacting a biological sample with a reagent that specifically binds to the polypeptide to form a reagent-polypeptide complex; and
 - 30 detecting the reagent-polypeptide complex.
 34. The method of claim 33 wherein the reagent is an antibody.
 35. A kit for detecting a polypeptide comprising the amino acid sequence shown 30 in SEQ ID NO: 2, comprising:

an antibody which specifically binds to the polypeptide; and

instructions for the method of claim 33.

5 36. A method of screening for agents which can modulate the activity of a human
dipeptidyl peptidase 8, comprising the steps of:

10 contacting a test compound with a polypeptide comprising an amino acid
sequence selected from the group consisting of: (1) amino acid sequences
which are at least about 62% identical to the amino acid sequence shown in
SEQ ID NO: 2 and (2) the amino acid sequence shown in SEQ ID NO: 2; and

15 detecting binding of the test compound to the polypeptide, wherein a test
compound which binds to the polypeptide is identified as a potential agent for
regulating activity of the human dipeptidyl peptidase 8.

37. The method of claim 36 wherein the step of contacting is in a cell.

38. The method of claim 36 wherein the cell is *in vitro*.

20 39. The method of claim 36 wherein the step of contacting is in a cell-free
system.

40. The method of claim 36 wherein the polypeptide comprises a detectable label.

25 41. The method of claim 36 wherein the test compound comprises a detectable
label.

30 42. The method of claim 36 wherein the test compound displaces a labeled ligand
which is bound to the polypeptide.

43. The method of claim 36 wherein the polypeptide is bound to a solid support.
44. The method of claim 36 wherein the test compound is bound to a solid support.
- 5
45. A method of screening for agents which modulate an activity of a human dipeptidyl peptidase 8, comprising the steps of:
 - 10 contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 62% identical to the amino acid sequence shown in SEQ ID NO: 2 and (2) the amino acid sequence shown in SEQ ID NO: 2; and
 - 15 detecting an activity of the polypeptide, wherein a test compound which increases the activity of the polypeptide is identified as a potential agent for increasing the activity of the human dipeptidyl peptidase 8, and wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human dipeptidyl peptidase 8.
- 20 46. The method of claim 45 wherein the step of contacting is in a cell.
47. The method of claim 45 wherein the cell is *in vitro*.
- 25 48. The method of claim 45 wherein the step of contacting is in a cell-free system.
49. A method of screening for agents which modulate an activity of a human dipeptidyl peptidase 8, comprising the steps of:
 - 30 contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO: 1; and

detecting binding of the test compound to the product, wherein a test compound which binds to the product is identified as a potential agent for regulating the activity of the human dipeptidyl peptidase 8.

5

50. The method of claim 49 wherein the product is a polypeptide.
51. The method of claim 49 wherein the product is RNA.
- 10 52. A method of reducing activity of a human dipeptidyl peptidase 8, comprising the step of:

15 contacting a cell with a reagent which specifically binds to a product encoded by a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 1, whereby the activity of a human dipeptidyl peptidase 8 is reduced.

53. The method of claim 52 wherein the product is a polypeptide.
54. The method of claim 53 wherein the reagent is an antibody.
- 20 55. The method of claim 52 wherein the product is RNA.
56. The method of claim 55 wherein the reagent is an antisense oligonucleotide.
- 25 57. The method of claim 56 wherein the reagent is a ribozyme.
58. The method of claim 52 wherein the cell is *in vitro*.
59. The method of claim 52 wherein the cell is *in vivo*.
- 30 60. A pharmaceutical composition, comprising:

a reagent which specifically binds to a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2; and

5 a pharmaceutically acceptable carrier.

61. The pharmaceutical composition of claim 60 wherein the reagent is an antibody.

10 62. A pharmaceutical composition, comprising:

a reagent which specifically binds to a product of a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 1; and

15 a pharmaceutically acceptable carrier.

63. The pharmaceutical composition of claim 62 wherein the reagent is a ribozyme.

20 64. The pharmaceutical composition of claim 62 wherein the reagent is an antisense oligonucleotide.

65. The pharmaceutical composition of claim 62 wherein the reagent is an antibody.

25

66. A pharmaceutical composition, comprising:

an expression vector encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2; and

30

a pharmaceutically acceptable carrier.

67. The pharmaceutical composition of claim 66 wherein the expression vector comprises SEQ ID NO: 1.
- 5 68. A method of treating a dipeptidyl peptidase 8 dysfunction related disease, wherein the disease is selected from, cancer, a CNS disorder or COPD comprising the step of:
10 administering to a patient in need thereof a therapeutically effective dose of a reagent that modulates a function of a human dipeptidyl peptidase 8, whereby symptoms of the dipeptidyl peptidase 8 dysfunction related disease are ameliorated.
- 15 69. The method of claim 68 wherein the reagent is identified by the method of claim 36.
70. The method of claim 68 wherein the reagent is identified by the method of claim 45.
- 20 71. The method of claim 68 wherein the reagent is identified by the method of claim 49.

Fig. 1

atgcggaagg ttaagaaaact gcgcctggac aaggagaaca ccggaagttg gagaagcttc
tcgctgaatt ccgagggggc tgagaggatg gccaccacccg ggaccccaac ggccgaccga
ggcgacgcag ccgcacaga tgacccggcc gcccgttcc aggtgcagaa gcactcgtgg
gacgggctcc ggagcatcat ccacggcagc cgcaagtact cgggcctcat tgtcaacaag
gcgcacccacg acttccagtt tgcagaag acggatgagt ctgggccccca ctccacccgc
ctctactacc tggaatgcc atatggcagc cgtgagaact ccctcctcta ctccgagatc
cccaagaaaag tgcggaagga ggcctgtg ctgtgtcct ggaagcagat gctggaccac
ttccaggcaca caccacca tggtgttac tcccgagagg aggagctact gcgaggcgc
aagcgctgg gctgtttcgg aatcaccttct tatgacttcc acagtgagag cggccttcc
ctctccagg ccaccaatg cctgttccac tgcaggatg gtggcaagaa tggctttag
gtgtccccca tgaagccact ggagatcaag actagtgtt ctggccacg catggacccc
aaaatctgcc ctgcccaccc tgccttctc tccctcatca ataacagcga cctgtgggtg
gccaacatcg agacaggcga ggagcggcgg ctgaccttct gccaccaagg tttatccaat
gtcctggatg accccaatgc tgcgggtgtg gccaccttcg tcatacagga agagttcgac
cgcttcaactg ggtactggtg gtgcacccaca gcctccctggg aaggttcaga gggcctcaag
acgctgcgaa tcctgtatga ggaagtcatg gagtccgagg tggaggtcat tcacgtcccc
tctcctgcgc tagaagaaaag gaagacggac tcgtatcggt acccccaggac aggacagcaag
aatcccaaga ttgccttgaa actggcttag ttccagactg acagccaggg caagatcgac
tcgaccagg agaaggagat ggtgcacccca ttcagctcgc tggccatggaa ggtggagtag
atcgccaggcc cgggtggac cgggatggc aaatacgcct gggccatgtt cctggaccgg
ccccagcagt ggctccagct ctgccttcc ccccccggcc tggatcgatccc gacacagag
aatgaggagc agcggctagc ctgccttcc gctgtccca ggaatgtcca gccgtatgt
gtgtacgagg aggttccacca cgtctggatc aatgttcatg acatcttcta tcccttcccc
caatcagagg gagaggacga gctctgttcc tcccgccca atgaatgcac gaccggctt
tgccatttgt acaaagtcat cgcctttta aaatcccagg gctacgatgg gagtgagccc
ttcagccccg gggaaagatga atttaagtgc cccattaaagg aagagatggc tctgaccagc
ggtgaatggg aggttttggc gaggcacggt tccaaagatct gggtaatga ggagaccaag
ctgggttact tccagggcac tatggatggc accacctcta cgtggtcagc
tatgaggcgg cggcggatg cgccttttta aaatcccagg gctacgatgg gagtgagccc
atgaggcaga acttcgacat gttcgttac cactacagca gcgtgagcac gcccctgc
gtgcacgtct acaagcttag cggcccccac gacgacccccc tgcacaagca gcccccttc
tgggttagca tgcgtggac agccagctgc ccccccggatt atgttccctcc agagatctt
cattttccaca cgcgtcgga tgcgtggatc tacggatgatca tctacaagcc ccacgcctt
cagccaggaa agaagcaccc gtcgttcc ttttatgtt gaggccccca ggtgcagctg
gtgaataact cttccaaagg catcaagttac ttgcgttca acacacttgc ctcctgggc
tacggcgtgg ttgtgattga cggcaggggc tcctgttca gagggtttcg gttcgaaggg
gccctgaaaa accaaatggg ccaggtggag atcgaggacc aggtggaggg cctgcagtt
gtggccgaga agtatggctt cattgtactt agccgagtcg ccatccatgg ctggccctac
gggggcttcc tctcgctcat ggggcttaatc cacaagcccc aggtgttcaa ggtggccatc
gccccgtcccc cggcgtccatgt ctggatggcc tacgacacag ggtacactga ggcgtacatg
gacgtccctg agaacaacca gacggctat gaggcgggtt ccgtggctt gcacgtggag
aagctgccccca atgagccaa cgcgttgcctt atcctccacg gttcccttggaa cgaaaacgtg
cactttttcc acacaaactt cctcgctcc caactgtatcc gaggcaggaa accttaccag
ctccagatct accccaacga gagacacagt attcgctgcc ccgagtcggg cgagcactat
gaagtcacgt tgctgcactt tctacaggaa tacctc

- 2/13 -

Fig. 2

MRKVKKLRLD KENTGSWRSF SLNSEGAERM ATTGTPTADR GDAAATDDPA ARFQVQKHSW
 DGLRSIIHGS RKYSGLIVNK APHDFQFVQK TDESGPHSHR LYLYLGMYPGS RENSLLYSEI
 PKKVRKEALL LLSWKQMLDH FQATPHHGKVY SREELLRER KRLGVFGITS YDFHSESGLF
 LFQASNSLFH CRDGGKNGFM VSPMKPLEIK TQCSGPRMDP KICPADPAFF SFINNSDLWV
 ANIETGEERR LTFCHQGLSN VLDDPKSAGV ATFVIQEEFD RFTGYWWCPT ASWEGSEGLK
 TLRILYEEVD ESEVEVIHVP SPALEERKTD SYRYPRTGSK NPKIALKLAE FQTDSQGKIV
 STOEKELVQP FSSLFPKVEY IARAGWTRDG KYAWAMFLDR PQQWLQLVLL PPALFIPSTE
 NEEQR LASAR AVPRNVQPYV VYEEVTNVWI NVHDIFYPFP QSEGEDELCF LRANECKTGF
 CHLYKVTAVL KSQGYDWSEP FSPGEDEFKC PIKEEMALTS GEWEVILARHG SKIIVVNEETK
 LVYFQGKDT PLEHHLYVVS YEAAGEIVRL TTPGFSHSCS MSQNFDMFVS HYSSVSTPPC
 VHVVYKLSGPD DDPHLHKQPRF WASMMEAASC PPDYVPPEIF HFHTRSVDRL YGMIYKPHAL
 QPGKKHPTVL FVYGGPQVQL VNNSFKGKY LRLNTLASLG YAVVVIDGRG SCQRGLRFEG
 ALKNQMGOVE IEDQVEGLQF VAEKYGFIDL SRVAIHGSY GGFLSLMGLI HKPQVFKVAI
 AGAPVTWMA YDTGYTERYM DVPENNQHGY EAGSVALHVE KLPNEPNRLL ILHGFLDENV
 HFFHTNFLVS QLIRAGKPYQ LQIYPNERHS IRCPESGEHY EVTLLHFLQE YL

Fig. 3

MAAAMETEQLGVEIFETADCEENIESQDRPKLEPFYVERYWSQL
 KKLADTRKYHGYMMAKAPHDFMFVKRNDPDGPHSDRIYVLA MGENRENTLFYSEIPK
 TINRAAVLMLSWKPLLDLFQATLDYGMYSREEELLRERKRIGTVGIASYDYHQGSGTFL
 FOAGSGIYHVKDGGPQGFTQQPLRPNLVETSCPNIRMDPKLCPADPDWIAFIHSNDIWI
 SNIVTREERRLTYVHNELANMEEDARSAGVATFVLQEEFDYRSGYWWCPKAETTPSGGK
 ILRILYEEENDESEVI IHVTSPLMELTRRADSFRYPKTGTANPKVTFKMSEIMIDAEGRI
 IDVIDKELIOPFELFEGVEYIARAGWTEPGKYAWSILLDRSOTRLQIVLISPFLFIPV
 EDDVUMERQRLIESVPDSVTPLIYEETTDIWINIHDIFHVFPQSHEEEIEFIFASECKT
 GFRHLYKITSILKESKYKRSSGGLPAPSDFKCPIKEIAITSGEWEVILGRHGSNIQVDE
 VRRLVYFEGTKDSPLEHHLYVVSYVNPGEVTRLTDRGYSHSCCISQHCDFFISKYSNQK
 NPHCVSLYKLSSPEDDPTCKTKEFWATILDSAGPLPDYTPPEIFSFESTTGFTLYGMLY
 KPHDLQPGKKYPTVLFYGGPQVQLVNNRFKGVKYFRNLNTLASLGYVVVVIDNRGSCHR
 GLKFEGAFKYKMGQIEIDDQVEGLQYLASRYDFIDLDRVGIHGWSYGGYLSLMALMQRS
 DIFRVAIAGAPVTIWFYDTGYTERYMHPDQNEQGYYLGGSVAMQAEKFPSEPNRLLL
 HGFLDENVHFAHTSILLSFLVRAGKPYDLQIYPQERHSIRVPESGEHYELHLLHYLQEN
 LGSRIAALKVI

Fig. 4

GAGGTTCAAGAGGCTCAAGACGCTGCGAATCCTGTATGAGGAAGTCGATGAGTCGAGGT
 GGAGGTCAATTACGTCCCTCTCCTGCGTAGAAGAAAAGGAAGACGGACTCGTATCGGTA
 CCCCAGGACAGGCAGCAAGAATCCCAAGATTGCCCTGAAACTGGCTGAGTTCCAGACTGA
 CAGCCAGGGCAAGATCGTCTGACCCAGGAGAAGGAGCTGGTGAGCCCTTCAGCTCGCT
 GTTCCCGAAGGTGGAGTACATGCCAGGGCCGGGTGGACCCGGGATGGCAAATACGCCCTG
 GGCCATGTTCCCTGGACCGGCCCCAGCAGTGGCTCCAGCTCGTCTCCCTCCCCCGCCCT
 GTTCATCCCGAGCACAGAGAATGAGGAGCAGCGGCTAGCCTCTGCCAGAGCTGTCCCCAG
 GAATGTCCAGCCGTATGTGGTGTACGAGGAGGTCAACACGTCCTGGATCAAATGTTCATGA
 CATCTCTATCCCTCCCCAATCAGAGGGAGAGGAGCAGCTCTGCTTCTCCCGCCTCAA
 TGAATGCAAGACCGGCTCTGCCATTGTACAAAGTCACCGCCTTAAATCCAGGGC
 TACGATTGGAG

- 3/13 -

Fig. 5

GGGGAGGTTTGGCGAGGCACGGCTCCAAGGGACCAAGGACACGCCGCTGGAGCACAC
 CTCTACGTGGTCAGCTATGAGGCGGCCGGAGATCGTACGCCCTCACCACGCCGGCTTC
 TCCCATAGCTGCTCCATGAGCCAGAACCTCGACATGTTGTCAGCCACTACAGCAGCGTG
 AGCACGCCGCCCTGCGTGCACGCTACAAGCTGAGCGGCCAGCTGCCCTGGATTATGTT
 CCTCCAGAGATCTTCCATTTCACACGCCCTCGGATGTCGGCTCTACGGCATGATCTAC
 AAGCCCCACGCCCTGCAGCCAGGGAAAGAACCCCCACCGTCTCTTGTATATGGAGGC
 CCCCAGGTGCAGCTGGTGAATAACTCCTTCAAGGCATCAAGTACTTGCGGCTCAACACA
 CTGGCCTCCCTGGCTACGCCGTGGTTGATTGACGGCAGGGCTCTGTCAGCGAGGGC
 TTCGGTTCGAAGGGGCCCTGAAAAAAACAAATGGGCCAGGTGGAGATCGAGGACCAGGTGG
 GAGGGCTCGAGTCCGTGAGCAGAAGTATGGCTCATCGAACCTGAGCCGAGTTGCA
 TCCAATGGCTGGGCTACGGGACTTACTCTCGCTATGGGGTAATCCACAAGCCCAGG
 GGGTCAGGGCAATCGCAGCTGGGCCACGGCTGGATGGCCAAGAAACAGCGTACACT
 GAGCGGAAGGGACGGCCGGAAAACAGACAGGAAAGGGAGTCCGGGGCCGCTCAGG
 GAGAAAGTGCCACGAAGCCACAGCAGGGATCACACAGGGCTGGGACACGGGGGGTT
 CCACAAAAACACGACACAAGGAGACGGGAAACACCAACAACAATACCAAGGAACAATGAGGC
 GCAGGGAAACGACACGAGAACACATGGACGCGACGAACAAGCAATGCGACAGCAGA
 GACGCAGGACGACCAGGACGCCAGTTGCCACGGTCCCTCGCGCGAGCGACCAGN

Fig. 6

GCTCGGATGTGCGGCTCTACGGCATGATCTACAAGCCCCACGCCCTGAGCCAGAGAAGA
 AGCACCCCACCGCTCTTGTATATGGAGGCCCTGGTGCAGCTGGTGAATAACTCCT
 TCAAAGGCATCAAGTACTTGGGCTCAACACACTGGCCTCCCTGGCTACGCCGTGGT
 TGATTGACGGCAGGGCTCTGTCAGCGAGGGCTTCGGTTGCAAGGGGCCCTGAAAAAC
 AAATGGGCCAGGTGGAGATCGAGGACCAAGGGCTTCAGTTCAGTTGCGCAGTTC
 ATGGCTTCATCGACACTGAGCCAGTGGCCATCCATGGCTGGTCTACGGGGCTTC
 TCGCTCATGGGCTAATCCACAAGCCCCAGGTGTTCAAGGTGGGCACTCGGGGTG
 GTCACCGTCTGGATGGCTACGACACAGGGTACACTGAGCGTACATGGACGTCC
 AACACAGCACGGCTATGAGGCGGGTCCGTGGCCCTGCACGTGGAGAAGCTGCC
 GAGCCCAACGATTGCTTATCTCCACGGTCCCTGGACAAAAGTGCACTTTTCC
 AAATTCTCGTCTCCAACTGATCCGAGCAGGGAAACCTTACAGCGTGGATCAAC
 GAGAGAACAGTTGCTGCCAGTCGGGACATATGAGTCGTTGACTCTAAGGAA
 CTCTGACCTGCACGGACGCCATTA

Fig. 7

AAAGCAGAGCTGTCCTCTCCCTGATTGGGGAAGGGATAGAAGATGTCATGAACATT
 GATCCAGACGTTGGTGACCTCTCGTACACCACATCGCTGGACATTCTGGGACAGC
 TCTGGCAGAGGCTAGCCGCTGCTCCTCATTCTCTGTCCTGGGATGAACAGGGCGGGGG
 GAGGAGGACGAGCTGGAGCCACTGCTGGGCCGGTCCAGGAACATGGCCCAAGCGT
 GCCATCCCGGGTCCACCCGGCCCTGGCGATGTACTCCACCTTGGGAACAGCGAGCT
 GGGCTGCACCAAGCTCTCTGGGTGAGACGATTTGCCCTGGCTGTCAGTCTGGAA
 CTCAGCCAGTTCAAGGCAATCTGGGATTCTGCTGCCCTGTCCTGGGTACCGATACGA
 GTCCGTCTTCTTCTAGCGCAGGAGAGGGGACGTGAATGACCTCACCTCGGACTC
 ATCGACTTCTCATACAGGATTGCA

- 4/13 -

Fig. 8

ATTACATCTATTTAAAGGAAAGCAAATATAACGATCCAGTGGTGGCTGCCCTGCTCCA
 AGTGATTCAAGTGCCTATCAAAGAGGAGATAGCAATTACCGAGTGGTGAATGGGAAGTT
 CTTGCCGGCATGGATCTAATATCCAAGTTGATGAAGTCAGAAGGCTGGTATATTTGAA
 GCCACCAAAGACTCCCCCTTAGAGCATCACCTGTACGTAGTCAGTAAATCCTGGA
 GAGGTGACAAGGCTGACTGACCGTGGCTACTCACATTCTGCTGCATCAGTCAGCACTGT
 GACTTCTTATAAGTAAGTATAGTAACCAGAAGAACCTCACACTGTGTGTCCTTACAAG
 CTATCAAGTCCTGAAGATGACCCAACCTGAAAACAAAGGAATTGGGCCACCATTG
 GATTCAAGTCCTCTTCTGACTATACCTCAGAAATTTCCTCTTGAAAGTACT
 ACTGGATTACATTGTATGGATGCTCTACAAGCCTCATGATCTACAGCCTGGAAAGAAA
 TATCCTACTGTGCTGTTCATATATGGTGGCTCAGGTGCAGTTGGTGAATAATGGTTT
 AAAGGAGTCAGTATTCGGCTTGAATACCCCTAGCCTCTAGGGTATGTGGTTGAGTG
 ATAGACAAACAGGGGATCCTGCAACCGANGGCTTAAATTGAAGGCGCTTAAATATAAA
 ATGGTCAAATAGAAATTGACGATCAGGTGGGA

Fig. 9

AAAGCAGAGCTCGCTCTCCCTCTGATTGGGGAAAGGGATAGAAGATGTCAACATT
 GATCCAGACGTTGGTGACCTCCCTGACACCACATACGGCTGGACATTCTGGGACAGC
 TCTGGCAGAGGCTAGCCGCTGCTCCCTCAATTCTCTGTGCTCGGGATGAACAGGGCCGGGG
 GAGGAGGACGAGCTGGAGCCACTGCTGGGCGGTTCCAGGAACATGGCCAGGCATTT
 GCCATCCGGGTCACCCGGCCCTGGCGATGTACTCCACCTCGGAAACAGCGAGCTGAA
 GGGCTGCACCAGCTCCTCTCCTGGGTCGAGCAGTCTTGCCCTGGCTGTCAGTCTGGAA
 CTCAGCCAGTTCAAGGCAATCTGGGATACTGCTGCCTGTCCTGGGTACCGATACGA
 GTCCGGTCTCCTCTCTAGCGCAGGAGAGGGGACGTGAATGACCTCACCTCGGACTC
 ATCGACTTCCTCAT

Fig. 10

CGGCTCCAAGGGACCAAGGACACGCCGTGGAGCACCACTCTACGTGGTCAGCTATGA
 GGCAGCCGGCGAGATCGTACGCCCTCACACGCCGCTTCTCCATAGCTGCTCCATGAG
 CCAGAACCTCGACATGTTGTCAGCCACTACAGCAGCGTGAAGCACGCCGCTGCGTGCA
 CGTCTACAAGCTGAGCGGGCCCGACGACGACGACCCCCCTGCACAAGCAGCCCCGCTTCTGGC
 TAGCATGATGGAGGGCAGCCAGCTGGGGGGGGATTAATGTTCCCTCAGAGATCTTCCATT
 CCACACCGCCTGGATGTGCGGCTCTACGGCATGATCTACAAGCCCCACGCCCTGCGAGCC
 AGGGAGAAGCACCCCACCGCCTCTTTGTATATGGAGGGCCCCAGGTGCAGCTGGTGA
 ATAACCTCTCAAAGGCATCAAGTACTTGGGCTCAACACACTGGGCTCCCTGTGGCTAC
 GCGTGGTTGTGATTGACGGCAGGGGCTCTGTCAAGCGAGGGCTTGGTGCAGTTCGT
 CTGAAAAACCAATGGGCCAGTGTGGAGATCGAGGACCTGGTGGAGGGCCTGCAAGTTCGT
 GGCGAGAAGTATGGCTCTGACTGAGGCCAGTTGGCTCCCTGGCTGTCCATACGGGGC
 TTCTCTCGCTCATGGGCTATCCACAGCCCGTGTCCGTGGCTCGCGGTGCCGGCCCTGG
 TTGCTACGACCGGTCTGGCTGTGGAC

- 5/13 -

Fig. 11

GCGGCTGTCGCCCCCGCTCCGCCACTCCGGGGTCGAGTCGGGGCATGGAGCCGCG
 ACCGTGAGGCAGCGCTGGACCCGGGACCTGCCAGTCCGGCGCCGCCACGTCCC
 GGTCTGTGTCACGCCCTGAGCTGGAAATGGAGGCTCTGGACCCCTTAAAGGCACCC
 CTGCCCTCCTGAGGTCAAGCTGAGCGGTTAATCGGAAAGGTTAAAGAAACTGCGCCTGGACA
 AGGAGAACACCGGAAGTGGAGAAGCTCTCGCTGAATTCCGAGGGGCTGAGAGGATGG
 CCACCAACGGGACCCCAACGGCCGACCGAGGCGAGCAGCCAGAGATGACCCGGCG
 CCCGCTTCAGGTGCAAGCAGCTCGTGGACGGGCTCCGGAGCATCATCCACGGCAGCC
 GCAAGTACTCGGGCCTCATTTCAACAAGCGCCACGACTTCCAGTTGTGAGAAGA
 CGGATGAGTCTGGGCCCCACTCCCACCGCCTACTACCTGGGAATGCCATATGGCAGCC
 GAGAGAACCTCCCTACTCTGAGATTCCAAGAAGGCTGGAAAGAGGCTCTGCTGC
 TCTTGTCTGAAAGCAGATGCTGGCATATTCCAGGCCACGGGCCCACATGGGGCTA
 CTCTCGGAGGAGGAGCTGCTGGGAGCGGAAACGCCCTTCCAGGCCACAGCCTTCTCCAC
 CCTACGACTTCAACAGCAGAGTGGCTCTTCCCTTCCAGGCCACAGCCTTCTCCAC
 TGGCGGACGGCGGAAGAACGGCTCATGGGTGCCCCATGAACCGTGGGAATTCACACCC
 AAGGGACAGGGCCCCGAGTGGAGCGAAAATCTGCCGGCACCAGCTATGCGTATATGCCGT
 CAAACAACAACAGGCACCCGTGGGTGCCCCATCTAACACGGGACGAAACGAGGGACC
 CTCCGCCAAGGGATCACGCCAGAGACCCAACCTAGTGGGGGACACTGCAGAGACAA
 CCAACACGAATCGTACACGTGGCGACCCCGCAGACAGAAGCAACACAGCAACGAAGA
 AGCAGACACAGGGCGCAAGCCGACCTAGACAGAGCAGACCGCAGGGTACGAGCACAA
 ACATCTGAAGACCGCAGCCACCGGCCCG

Fig. 12

GAGTCAAGTATTCCCGCTTGAATACCCCTAGCCTCTAGGTTATGTGGTTAGTGATA
 GACAACAGGGGATCCGTACCGAGGGCTAAATTGAAAGGCCCTTAAATATAAAATG
 GGTCAAATAGAAATTGACGATCAGGTGGAAAGGACTCCAATATCTAGCTTCTGATATGAT
 TTCATTGACTTAGATCGTGGGCATCCACGGCTGGCTATGGAGGATACTCTCCCTG
 ATGGCATTAAATGAGGGTCAGATATCTCAGGGTTGCTATTGCTGGGCCCCAGTCACT
 CTGTGGATCTTCTATGATAACAGGATACACGGAACGTTATGGGTACCCCTGACCAGAAT
 GAACAGGGCTATTACTAGGATCTGGCCATGCAAGCAGAAAAGTCCCTCTGAACCA
 AATCGTTACTGCTTACATGGTTCTGGATGAGAATGTCATTTGCACATACCAAGT
 ATATTACTGAGTTTTAGTGGAGGGCTGGAAAGCCATATGATTACAGATCTATCCTCAG
 GAGAGACACAGCATAAGAGTCTGAATCGGGAGAACATTATGAACTGCATTTGCAC
 TACCTCAAGAAAACCTTGGATCACGTATTGCTCTAAAAGTGTATAATTGACCT
 GTGTAGAACTCTGGTATAACTGGTATTGACCAATGAGGACGTGTAATCGAAGCGA
 AAACCCAGAAATGGTCATGCCATTGTTACCTGCAATTGTTAGCATTACTCTGGAA
 ATTAATGTTGGTGCCATGCCGGCTTACGGGTGGGGAAATTAAACTTAAACCCCATG
 TGCTAATCAATGTTCTGGAAACCGAGTCTAGATTCTAAAAAAACCGAGATT
 CTTCCAGAGGGCGCAAGGGGCCACAGGGCTTAAAGGCTGGAGAGACCGTCAATGTG
 CAGTGTGCAAACCTTCTGGAGAGAATTGATGAGGGAG

- 6/13 -

Fig. 13

GGCTTCTGCCATGTGTACAAGTCACCGCCGTTAAAATCCCAGGGTACGATGGGAGT
 GAGCCCTTCAGCCCCGGGAAAGATGAATTAAAGTCCCCATTAGGAAGAGATGGCTCTG
 ACCAGCGGTGAATGGGAGGTTTGGCGAGGCACGGTCTCAAGATCTGGTCAATGAGGAG
 ACCAAGCTGGTGTACTTCCAGGGACCAAGGACACGCCGCTGGAGCACCACCTCTACGTG
 GTCAGCTATGAGGCCGGCGAGATCGTACGCCCTAACCAGGCCGGTCTCCCATAG
 CTGGCTCATGAGCCAGAACCTTCGACATGTTCTGTCAGCCACTACAGCAGCGTGAGCACGC
 CGCCCTGCGTGCACGCTAACAGCTGGAGCTGGCCCCGANGAGGAACCCCTGCACAAGC
 AGCCCGGGTCTGGGCTAGCATGATGGAGGCAGCCAGGTCCCCGGGATTATGTTCTC
 CAGAGATCTTCCATTCCACCGCGCTGGATGTCGGTCTACGGCATGATCTAGAACCC
 CAAGCTGGAGGCCAGGAAGACCAACGCCCTTGGATTGGGCCCCAGGTGCCGTGG
 GGCTAATCTTCAAGGGCTCAGGACATGCGGGTACACAATGGGCTCCCTGGGTACGCCGG
 AAGGAGATGCCGGAGGGGGCCGGACAGGGGGCAGGGCAGGAAAGGGCCGAGAAAAATTGGG
 CGGGAACCGGACTGGAGGGCAAAATTGGCACACAGTGGTAAAGCGAGAAAGGGACA
 CCAAGCGAGGGACAGGAGGACCTACAAGTGGGTACAACCCCGTAGAGAGAGAACCG
 ACCCACGGGATGCAAAGAACACGAGAACCAACAAATCAAAGTAGCGAGCGCACGAAAC
 AATGCAAGCGTAAGTCTTCTCGCTGCCCTCGCTCGCGCCGCTGTCCTGCTGTTGGT
 GGAGAGGAG

Fig. 14

TGGCGAATTGGCACGGAGGGGAGGTTTGGCGAGGCACGGCTCCAAGGGCACCAAGGAC
 ACGCCGCTGGAGCACCCACCTCTACGTGGTCAGCTATGAGGCCGGCGAGATCGTACGC
 CTCACACGCCGGCTTCTCCATAGCTGCTCATGAGCCAGAACCTCGACATGTTCTGTC
 AGCCACTACAGCGTGAGCACGCCCTGCGTGCACGCTACAAGCTGAGCGGCC
 GACGACGACCCCTGACAAGCAGCCCCCTCTGGGCTAGCATGATGGAGGCAGCCAGC
 TGCCCCCGGATTATGTTCTCCAGAGATCTTCCATTCCACACGCGCTGGATGTGGG
 CTCTACGGCATGATCTACAAGCCCCACGCCCTGCAAGCCAGGGAGAACGCACCCACCGTC
 CTCTTGTATATGGAGGCCCC

Fig. 15

CAACAGGGGATCCTGTCACCGAGGGCTTAAATTGAAAGGCCCTTAAATATAAAATGGG
 TCAAATAGAAATTGACGATCAGGTGGAAGGACTCCAATATCTAGCTTCTGATATGATT
 CATTGACTTAGATCGTGTGGGATCCACGGCTGGTCTATGGAGGATACCTCTCCCTGAT
 GGCATTAATGCAGAGGTCAAGATATCTCAGGGTTGTATTGCTGGGCCCCAGTCACTCT
 GTGGATCTTCTATGATAACAGGATACAGGAACGTTATATGGGTCACCCTGACCAGAAATGA
 ACAGGGCTATTACTTAGGATCTGTGGCATGCAAGCAGAAAAGTCCCCCTGAAACCAA
 TCGTTACTGCTTACATGGTTCTGGATGAGAATGTCATTTGCACATACAGTAT
 ATTACTGAGTTTTAGTGGGGCTGAAAGCCATATGATTACAGATCTACCTCAGGA
 GAGACACAGCATAAGAGTTCTGAATCGGGAGAACATTATGAACTGCATTTGCACCA
 CCTTCAAGAAAACCTGGATCACGTATTGCTCTAAAGTGTATAAATTGACCTGT
 GTAGAACTCTGGTATACACTGGCTATTAAACCAATGAGGAGGTTAATCAACAGAAA
 ACACAGAATTGATCATCACATTGATACCTGCCATGTAACATCTACTNCTGAAAATAA
 TGTGGTGCCATGCAG

- 7/13 -

Fig. 16

GTCTACGGGGCTTCCCTCGCTCATGGGCTAATCCACAAGCCCCAGGTGTTCAAGGTG
 GCCATCGGGGTGCCCCGGTCACCGTCTGGATGGCCTACGACACAGGGTACACTGAGCGC
 TACATGGACGTCCCTGAGAACAAACCGACGGCTATGAGGCGGGTCCGGCTCTGCAC
 GTGGAGAAGCTGCCAATGAGCCAACCGCTTGTTATCCTCACGGCTTCCCTGGACGAA
 AACGTGACTTTCCACACAAACTTCTCGTCTCCAACGTGATCCGAGCAGGGAAACCT
 TACCAGCTCAGATCTACCCAAACGAGAGACACAGTATTGCTGCCAGTCGGCGAG
 CACTATGAAGTCACGTTGCTGACTTTACAGGAATACCTCTGAGCCTGCCACCGGGA
 GCCGCCACATCACAGCACAAGTGGCTGAGCCTCCGCCAGGCTGGCAAGCCCCGAGGA
 AGTGGCCCGGGCCCCAGTGGGACTTTGCTCCGCCAGGCTGGCAAGCCCCGAGGA
 GCCGCTGCCCTTACCGCCGACGCCCTTATCCTTTAAACGCTCTGGGTTATGT
 CCGCTGCTCTGGTGTGGAGACAGAGAGATGGTGTCTGGGCCAGCCCCCTCTC
 CCCGCCCTCTGGAGGAGGAGGTACACGCTGATGGGACTGGAGAGGCAGAAGAGACTC
 AGAGGAGGGGGCTGCTTCCCTGGGCTCCTGTGACCTCTCAGTCCCCCTGGCCGGGCA
 GGCGGTCCCAGGCCAAGCTGCTTGTGGTCCCCCGGGCGTTCCCACTTGTGTTGGG
 TTGGTGGGGGGATTCCATTAAAGAGGGGGGGGGCGTGTCCC

Fig. 17

TGCCAACAGCCTCTGGGAGGTTCAAGAGGCCCTCAAGACGCTGCGAATCTGTATGAG
 GAAGTCGATGAGTCCGAGGTGGAGGTCAATTACGTCAGTCCCCCTCTCGCCTAGAAGAAAGG
 AAGACGGACTCCATCGGTACCCAGGACAGGCAGAGAATCCAAGAATGCCCTTGAAA
 CTGGCTGAGTCCAGACTGACAGCCAGGGCAAGATCGTCTCGACCCCCAGAGAAAGAGCTG
 GTGCAGCCCTCAGCTGCTGTTCCGAAAGGTGGAGTACATGCCAGGGCCGGGTGGACC
 CGGGATGGCAAATACGCTGGGCCATGTTCTGGACCGGCCCCAGCAGTGGCTCCAGCTC
 GTCCCTCCTCCCCGGCCCTGTTCATCCGAGCAGAGAATGAGGAGCAGCGCTAGCC
 TCTGGCAGCTGTCNCAGGAATGTCCAGCGTAT

Fig. 18

GCTCTTACCGACTTGGCTCTTGGGTCTTGCTCTTGGGTCTTGCTCATGGT
 TCCCCCTATGAAACCCGCTGAAATCAAGACCCAGTGTCTCAGGGCCCCGGATGGACCCAAA
 ATCTGCCCTGCCGACCTCTGCCCTTCTCCTCATCAATAACAGCGACCTGTGGGTGGCC
 AACATCGAGACAGGCAGGGAGCAGGGCTGACCTTCTGCCACCAAGGTTATCCAATGTC
 CTGGATGACCCCAAGTCTGGGTGTGGCACCTTCTGTCATACAGGAAGAGTTCGACCGC
 TTCACTGGGTACTGGTGGTCCCCACAGCCTCTGGAAAGGTTCAAGAGGCTCAAGACG
 CTGCGAATCTGTATGAGGAAGTCGATGAGTCGAGGTGGAGGTCAATTACGTCCACTCT
 CCTGCGCTAGAAGAAAGGAAGACGGACTCGTA

Fig. 19

CGTCGTGTAATCCGTACAATGAACAAACAGCATATTGAAACCACAGTGACTAACGCGATG
 AGTCGACAGCATTGTCAGGTCTGCTCACCTTCCCCGGGGCTGAAGGGCTCACTCCAAT
 CGTAGCCCTGGGATTAAACAGGGGTGACTTTGATCAAATGGCAGAAGCCGGTCTTGC
 AATTCAATTGGCGCGAGAAAGCAGAGCTCGTCCTCTCCCTGATTGGGGAAGGGATAG
 AAGATGTCAATGACATTGATCCAGACGTTGGTGACTCTCTGTCATACACACATACGGCTGG
 ACATTCTGGGACAGCTGGCAGAGGCTAGCCGTGCTCTCATTCCTGTGACTCCACCTC
 ATGAAACAGGGCCGGGGGAGGAGGACGAGCTGGAGGCCACTGCTGGGGCCGGTCCAGGAAC
 ATGGCCCAGGCGTATTGCAATCCGGGTCCACCCGGCCCTGGCGATGTAACCCACCTC
 GGGACAGCGAGCTGAAGGGCTGACCCAGCTCCTCTGGGTGAGAGGAGATCTGCCC
 TGGCTGTCAGTCTGG

- 8/13 -

Fig. 20

GATCTTGCCTGGCTGTCAGTCTGGAACCTCAGCCAGTTCAAGGCAATCTTGGGATTCTT
 GCTGCCTGCTGGGATACCGATACGAGTCCGCTTCCTTCTTAGCGCAGGAGAGGG
 GACGTGAATGACCTCCACCTCGGACTCATCGACTTCCTCATACAGGATTCCGAGCGTCTT
 GAGGCCCTCTGAACCTTCCAGGAGGCTGTGGGGCACCCAGTACCCAGTGAAGCGGTC
 GAACTCTCCTGTATGACGAAGGTGGCACACCCGAGACTTGGGTATCCAGGACATT
 GGATAAACCTTGGTGGCAGAAGGTAGCCGCGCTCCCGCT

Fig. 21

AACGGGTGATGCGGTTGGTTAAGAAACTGCGCCTGGACAAGGAGAACACCGGAAGTTGGA
 GAAGCTTCTCGCTGAATTCCAGGGGCTGAGAGGATGCCACCACGGGACCCAAACGG
 CCGACCGAGGCAGCGAGCCGCCACAGATGACCCGGCCCGCTTCCAGGTGCAGAAC
 ACTCGTGGGACGGGCTCCGGAGCATCATCCACGGCAGCCGAAGTACTCGGGCTCATTG
 TCAACAAGGCGCCCCACGACTTCCAGTTGTGAGAACAGGGATGAGTCTGGGCCCCACT
 CCAACCGCATACGGGT

Fig. 22

TTAATGCGGAAGGTTAAGAAACTGCGCCTGGACAAGGAGAACACCGGAAGTTGAGAAC
 TTCTCGCTGAATTCCAGGGGCTGAGAGGATGCCACCACGGGACCCAAACGGCCGAC
 CGAGGCAGCGAGCCGCCACAGATGACCCGGCCCGCTTCCAGGTGCAGAACACTCG
 TGGGACGGGCTCCGGAGCATCATCCACGGCAGCCGAAGTACTCGGGCTCATGTCAAC
 ATCGAGACAGGGAGGAGCAGGGCTGACCTTCTGCCACCAAGGTTATCCAATGTCTG
 GATGACCCCAAGTCTGCGGGTGTGGCACCTTCGTATACAGGAAGAGTTGACCGCTC
 ACTGGTACTGGGGTCCCCACAGCCTCTGGAAAGGTTAGCAGAGGGCTCAAGACGCTG
 CGAATCTGTATGAGGAAGTCGATGAGTCCGAGGTGGAGGTATTACGTACCCCTCCT
 GCGCTAGAAGAAGGAAAGACGGACTCGTATCGGTACCCCA

Fig. 23

AGGCTGTGGGCACCACCACTACCGTAGTGAAGCGGTCGAACCTTCTGTATGACGAAC
 TGGCCACACCCGAGACTTGGGTATCCAGGACATTGGATAAACCTTGGCAGAAGG
 TCAGCCGCGCTCTCGCTGTCTCGCATGTTGGCACCCACAGGTGCTGTTATGATG
 AAGGAGAAGAAGCAGGGTCCGAGGGCAGATTGGGGTCCATCCGGGGCCCTGAGCAC
 TGGGCTTGATTCCAGCGTTTCATAGGGGACACCAGTGAAGCCGTTCTGCCCGCTCG
 CGGAGTGGAAAGGGCTGTTGCTGGCCTGGAAGAGGAAGTGGCAGTCTCCGCTGTGGAG
 TCGTAGGAGGTGATGCCAAGACCCCAAGCGTTCCGCTCCCTCAGCAGC

Fig. 24

TTGTGGCTCAGTGCCAATAAAACTTTATTAACACAGGTGGTGGCCGATTGACC
 TGTGGCCGTAGGTGGCAACCCCTGGTTGTGCTAAGGGCTGGCACAGGGAGGTGCTC
 AGTAATCTGCTGCTGAATAAGCCAACAGTTGGGTGCTTAAGCCCTGCCAGATCATGC
 AGTCACTGGGAAGGCTGGCTCTGCCATCCCTTCCCGCAGGGTGTGCTG
 GCTGAGTGGGGGGCGACCAATGAACAAACAGCATATTGAACACACGTGACTAACGGC
 ATGAGTCGACAGCATTGTCAGGCTCTGCTCACCTCCCCGGGCTGAAGGGCTACTCC
 AATCGTAGCCCTGGATTAAACGGCGGTGACTTTGTACAAATGGCAGAAGCCGGTCT
 TGCATTGATTGGCGGGAGAAAGCAGAGCTCGTCTCCCTCTGATTGGGGAGGGAT
 AGAAGATGTCATGAACATTGATCCAGACGTTGGTGAACCTCCTCGTACACACATACGGCT
 CGGACATTCTGGGACAGCTCGCAGAGGCTAGCCGCTGCTCTGATTCTGTGCTCG

- 9/13 -

Fig. 25

AGATCGAGGACCAGGTGGAGGGCTGCAGTTCTGTGGCCGAGAAGTATGGCTTCATCGACC
 TGAGCCGAGTTGCCATCCATGGCTGGTCTACGGGGCTTCCCTCGCTCATGGGCTAA
 TCCACAAGCCCCAGGTGTTCAAGGTGGCCTACGGGGTGCCTCGGTACCGCTGGATGG
 CCTACGACACAGGGTACACCTGAGCGCTACATGGACTGTCTGAGAACAAACCAGCACGGC
 TATGAGGCGGGTCTCGTGGCCCTGCCCACGTTGTGAGAATGGCTGCCCAAATGAGCCC
 CAAACCCCGCCTTGCCTTAATCCNTCCCCACGTGGCTTTCCCTNTGTGTTAACCG
 GAAAAAAACGTTGCAACACTTATATTTTCGCCAAGCGAAGGCCAAAACAACGGTTTCC
 CCAATTACGGTGATTCCCTTCCCCCAGAACCTGGAAAATTCCCCGTAATGCCAG
 GGGGAAAAAAACCTTACCAAGCTCCAGATCTACCCCAACGAGAGACACAGTATTGCTGC
 CCCGAGTCGTGGCGAGCACTATGAAGTCACGTTGCTGCACTTTTCCCTAACAGGGGGG
 AAAATTAAACCCCTTCCCTGGAAAGGCCCTTGGCCCCCAA

Fig. 26

GACATAAAACCAAGAGCGTTAAAGAAAAGGATAAAAGGCGTCGGGGCGGTGAAGGCAG
 CGGCTCTCGGGCTGGCCAGCGCTGGCGGGACAAAGTGCCTCACTGGGGCCCGCGGGC
 CACTCAGTCCTCCCGCCTGGTCCCCCGGGAGGCTGCAGCCACTTGTGCTGTGATGTGG
 CGGCTCCCGGTGGCAGGCTCAGAGGTATTCCGTAGAAAGTGCAGCAACGTGACTTCAT
 AGTGCTCGCCGACTCGGGGCAGCGAATACTGTGCTCTCGTTGGGTAGATCTGGAGCT
 GGTAAGGTTTCCCTGCTCGGATCAGTTGGAGACGAGGAAGTTGTGAAAGGTTGCA
 CGTTTCTGTCAGGAAGCCGTGGAGGATAAGCAAGCGTTGGCTCATGGCAGCTTCT
 CCACGTGCAGGGCCACGGAACCCCGCTC

Fig. 27

GACATAAAACCAAGAGCGTTAAAAAAAGGATAAAAGGCGTCGGGGCGGTGAAGGCAGC
 GGCTCTCGGGCTGGCCAGCGCTGGCGGGACAAAGTGCCTCACTGGGGCCCGCGGGC
 ACTCAGTCCTCCCGCCTGGTCCCCCGGGAGGCTGCAGCCACTTGTGCTGTGATGTGGC
 GGCTCCCGGTGGCAGGCTCAGAGGTATTCCGTAGAAAGTGCAGCAACGTGACTTCATA
 GTGCTCGCCGACTCGGGGCAGCGAATACTGTGCTCTCGTTGGGTAGATCTGGAGCTG
 GTAAGGTTTCCCTGCTCGGATCAGTTGGAGACGAGGAAGTTGTGAAAGGTTGCA
 GTTTTCTGTCAGGAAGCCGTGGAGGATAAGCAAGCGTTGGCTCATGGCAGCTTCTC
 CACGTGCAGGGCCACGGAACCC

Fig. 28

TCTGGTCGTGCGTGGCATGGTGTGGACTTTGGGTGGCTGTGGCTTCTCCACGTGCAGG
 CCACGGAACCCGCTCATAGCCGTGCTGGTTGTTCTCAGTGGACGTCCATGTAGCGCTCA
 GTGTACCCCTGTGTCGTAGTGCCATCCAGACGGTGACCGGGCACCCCGCATGGCCACCTT
 GAACACCTGGGGCTTGTGGATTAGCCCCATGAGCGAGAGGAAGGCCCGTAGGACCAGCC
 ATGGATGGCAACTCGGCTCAGGTGATGAAGCCATACTCTCGGCCACGAACGTGCAGGCC
 CTCCACCTGGTCTCGATCTCCACCATGAG

- 10/13 -

Fig. 29

BLASTP - alignment of 181_Protein against trembl|AF221634|AF221634_1
 gene: "DPP8"; product: "dipeptidyl peptidase 8"; Homo sapiens dipeptidyl peptidase 8 (DPP8) mRNA, complete cds. //:gp|AF221634|11095188 gene: "DPP8";
 product: "dipeptidyl peptidase 8"; Homo sapiens dipeptidyl peptidase 8 (DPP8)
 mRNA, complete cds.
 This hit is scoring at : 0.0 (expectation value)
 Alignment length (overlap) : 840
 Identities : 61 %
 Scoring matrix : BLOSUM62 (used to infer consensus pattern)
 Database searched : nrdb_1_;

Q: 53 FQVQKHSWDGLRSIIHGSRKYSGLIVNKAPHDFQFVQKTDESGPHSHRLYYLGMPYGSRE
 F.V: : : SW. L: : : . : : RKY.G: : : KAPHDF.FV: : : D..GPHS.R: YYL.M. : : RE
 H: 35 FYVERYSWSQLKKLLADTRKYHGYMMAKAPHDFMFVKRNDPDGPHSDRIVYVLAMSGENRE

NSLLYSEIPKKVRKEALLLSWKQMLDHFQATPHGVYSREEELLRERKRLGVFGITSYD
 N: L. YSEIPK. : : : A:L:LSWK. : LD FQAT . : G:YSREEELLRERK:G..GI.SYD
 NTLFYSEIPKTINRAAVLMLSWKPLLDLFQATLDYGMYSREEELLRERK:RIGTVGIASYD

FHSESGLFLFQASNSLFHCRDGGKNGFMVSPMKPLEIKTQCSGPRMDPKICPADPAFFSF
 : H. SG.FLFQA. : : : H. : DGG..GF. . P: : P : : T.C.. RMDPK:CPADP : : F
 YHQGSGTFLFQAGSGIYHVKDGGPQGFTQQPLRPNLVETSCPNIRMDPKLCPADPDWIAF

INNSDLWVANIEETGEERRLTFCHQGLSNVLDDPKSAGVATFVIQEEFDRFTGYWWCPTAS
 I: : : D:W: : NI T EERRLT: . H. L: N: : D: : SAGVATFV:QEEFDR: : GYWWCP. A.
 IHSNDIWIISNIVTREERRLTIVHNELANMEEDARSAGVATFVLQEEFDRYSGYWWCPKAE

WEGSEGLKTLRILYEEVDESEVEVIHVPSPALEERKTDSYRYPRTGSKNPKIALKLAEFQ
 . S G K. LRLYEE DESEVE: IHV. SP. LE. R: . DS: RYP: TG: . NPK: . K: : E..
 TTPSGG-KILRILYEEENDESEVEIIHVTSPMLETRRADSFRTYKPTGTANPKVTFKMSEIM

-TDSQGKIVSTQEKELVQPFSSLFPKVEYIARAGWTRDGKYAWAMFLDRPQQWLQLVLLPP
 . D: : G: I: . : KEL: QPF. LF. VEYIARAGWT : GKYAW: : LDR. Q. LQ: VL: . P
 IDAEGRIIDVIDKELIQPFEILFEGVEYIARAGWTPEGKYAWSILLDRSQTRLQIVLISP

ALFIPSTENEEQRLASARAVPRNVQPYVYEEVTVNVINVHDIFYPFPQSEGEDELCFLR
 . LFIP : : R . . . : VP : V.P. : : YEE.T: : WIN: HDIF: FPQS. E: E: F:
 ELFIPVEDDVMERQRLLIESVPDSVTPLIYEETTDIWINIHDIFHVFPQSH-EEEIEFIF

ANECKTGFCHLYKVTAVLKSQGYDWSEPFSPGEDEFKCPIKEEMALTSGEWEVLARHGSK
 A: ECKTGF HLYK: T: : LK.. Y. S. P...: FKCPIKEE: A: TSGEWEVL. RHGS.
 ASECKTGFRHLYKITSILKESKYKRSSGGLPAPSDFKCPIKEEIAITSGEWEVLGRHGSN

IWVNEETKLVYFQGKTDPLLEHHLYVVSYEAAAGEIVRLTTPGFSHSCSMSQNFDMFVSHY
 I V: E : : LVYF: GTKD: PLEHHLYVVSY . GE: . RLT. G: SHSC. : SQ: D. F: S. Y
 IQVDEVRRLVYFEGTKDSPLEHHLYVVSYVNPGEVTRLTDRGYSHSCCISQHCDFFISKY

SSVSTPPCIVHVKLSGPDDPLHKQPRFWASMMEAASCPPDYVPPEIFHFHTRSDVRLYGS
 S: . . P CV: . : YKLS. P: DDP. K... FWA: : : : A. PDY. PPEIF. F: : : : . LYG
 SNQKNPHCVSLYKLSSPEDDPTCKTKEFWATILDSAGPLPDYTPPEIFSFESTTGFTLYG

< Prolyl oligopeptidase family (underlined) >

MIYKPHALQPGKKHPTVLFVYGGPQVOLVNNSPKGIKYLRLNTLASLGYAVVVVIDGRGSC
 M: YKPH LQPGKK: PTVLF: YGGPQVQLVNN. PKG: KY. RLNTLASLGY. VVVID. RGSC
 MLYKPHDLQPGKKYPTVLFYGGPQVQLVNNRFKGVKYFRLNTLASLGYVVVVVIDNRGSC

- 11/13 -

Fig. 29 (continued)

< active site: S residue >
QRGLRFEGALKNQMGQVEIEDQVEGLQFVAEKYGFIDLSRVAIHGWSYGGFLSLMGLIHK
.RGL:FEKA.K :MGQ:EI:DQVEGLQ::A.:Y.FIDL.RV.IHGWSYGG:LSLM.L:::
HRGLKFEGAFKYKMGQIEIDDQVEGLQYLASRYDFIDLDRVGIHGWSYGGYLSLMALMQR

PQVFKAIAGAPVTVWMAYDTGYTERYMDVPENNQHGYEAGSVALHVEKLPNEPNRLLL
.F:VAIAGAPVT:W: YDTGYTERY.M. P:.N:.GY .GSVA:..EK.P:EPNRLL:L
SDIFRVAIAGAPVTLWIFYDTGYTERYMGHPDQNEQGYYLGSVAMQAEKFPSEPNRLLL

< active site: D residue > < active site: H residue >
HGFLDENVHFFHTNFLVSQLIRAGKPYQLQIYPNERHSIRCPESGEHYEVTLHFLQEYL 892
HGFLDENVHF HT: L:S L:RAGKPY.LQIYP.ERHSIR.PESGEHYE: LLH:LQE L
HGFLDENVHFAHTSILLSFLVRAGKPYDLQIYPQERHSIRVPESGEHYELHLLHYLQENL 872

- 12/13 -

Fig. 30

HMM_Pfam - alignment of 181_Protein against pfam|hmm|DPPIV_N_term
 Dipeptidyl peptidase IV (DPP IV) N-termi -

This hit is scoring at : -72.9; E= 2.4e-07

Scoring matrix : BLOSUM62 (used to infer consensus pattern)

Q: 132 LSWKQMLDHFQATP-HHGVYSRE---EELLERKRLGVFgITSYDFHSESGLFLFQASNS
 H: 1 vtledifsgtfrpkdydinWIssedgeylyqdqdtlnli.vnyetgkttvllsdefnne
 LFHCR-----DGGKNGFMVSPMKPL-----EIKTQ-----CSG----
 D ... K ... :T G
 feasnksYeveiSpDlkylIstnyekrWRHSytasYiyiDlntgdvevpapeelgdDNY
 --PRMDPKICPADPAFFSFINNSDLWVANIEGEEERRLTFCQGLSNVLddpkSAGVAT
 P . . :F:..:L:V.. . :G.. : :T . G SN : G:..
 LlnkIqyatWSPNkGhklaFVrdNNLYvkknpsgpaqiTt..dGksndI....fNGipD
 FVIQEEFDRFTG-YWWCPTASwegseglktlRILYEEVDESEVEVIHVPSPALEE----
 :V.:EE. . . . WW.P... Y . . :SEV.VI..P . . .
 WYYEEEIILstdyAlWWSPDGd.....fLAYlrfnDseVPvieyPfYtddkvveie
 --RKTDSYRYPRTGSKNPKIALKLAEFQTdsqGKIVSTqeKELVQPFSSLFPKVEYIAR
 :T . . :YP:..G:..NP:..L :..... G. VST . . . :L . YI..
 dqYpetmeikYPKAGapNPtVklfvvnlad...gasvst..pveiplpanlasgdyYite
 AGWTR--DGKYAwAMFLDRPQQWLQLVLppalfipsteneEQRLASARAVPRNVQpyvV
 . W.. : :.A . :L:R.Q. L L. : V :N :
 VtWvtmknerla.VqwlnRdQnisvlslc.....Dtasssktwnvvnknre...h
 YEEVTNVWINVHD-IFYPFPQSEGE-DELCFLRANEcKTG--FCHLYKVTAVLKSqgydw
 . EE .. W:...: . FP . . :L . : :G : HL ... KS
 ieestgWvetfnpslPvfpIglsrlkeyYliisd.rdgkYkHlayyeldgks.....
 sepfspgedefkcpikEEMALTSGEWEVLarhgsK--IW-VNEETKLVVFQGKDTPLEH
 E.:ALT.G.WEV. . . I V:::T . VYF .T:.. E.
epialTkGnWEvt.....ngiilgvdsktltyvFlatekgsgeR
 HLYVVSYEAAGE-----IVRLTT--PGFsHSCSMSQNFDMFVSHYSSVSTPPCVHVK
 HLY :S:... . : . . G: :S.S.S.N
 hlysislkgetsktC1scqldserDCcg.ySasFSsnakYyiltsyGPgvPtigtlhs
 LSGP-----DDDPPLH--KQPRFWASMMEEAAscppdyVPPEIFHFHTR-----SD
 D.:
 skdekveksdlGkvkdkelrtLEdNealkkalknnyq.....1PskefkkkklpdDFadg
 VRLYGMIIYKPHALQP-GKKHPTVLFVYGGPQVQLVNN 683
 :.L KP . . P
 itlnyqmkPanFdpLsKKYPvLffvYGGPgSQqVtk 566

- 13/13 -

Fig. 31

HMMER - alignment of 181_Protein against pfam|hmm|Peptidase_S9
Prolyl oligopeptidase family

This hit is scoring at : 36.8; E= 4.3e-10

Scoring matrix : BLOSUM62 (used to infer consensus pattern)

Q: 692 RLNTLASLGYAVVVIDGRGSCQRGLRFEGALKNQMGQVEIEDQVEGLQFVaEKYGFIDLS
.....L . G ...V:D RG. : G ::: A . . . : E..D :K.G:...
H: 10 vasllnhrGgiyAvvdirGgGeyGqkwheagtrrlkknefnDfiaAAeyl.sklGYtspk

RVAIHGSYGGFL 764
R:AI.G S GG.L
riaifGgSnGG1L 81

Prolyl endopeptidase family serine proteins. region from residue 734 to 764. Source: [blocks database]

SEQUENCE LISTING

<110> Bayer AG

<120> REGULATION OF HUMAN DIPEPTIDYL PEPTIDASE 8

<130> Lio 298

<150> USSN 60/268,863

<151> 2001-02-16

<160> 28

<170> PatentIn version 3.1

<210> 1

<211> 2676

<212> DNA

<213> Homo sapiens

<400> 1

atgcggaaagg ttaagaaaact gcgcctggac aaggagaaca ccggaagttg gagaagcttc 60

tcgctgaatt ccgagggggc tgagaggatg gccaccacgg ggaccccaac ggccgaccga 120

ggcgacgcag ccgccacaga tgacccggcc gcccgttcc aggtgcagaa gcactcgtgg 180

gacgggctcc ggagcatcat ccacggcagc cgcaagtact cgggectcat tgtcaacaag 240

gcgcacccacg acttccagtt tgtgcagaag acggatgagt ctggggccca ctcccacccgc 300

ctctactacc tggaaatgcc atatggcagc cgtgagaact ccctctctta ctccgagatc 360

cccaagaaaag tgccggaaagg ggccctgtcg ctgtgtccct ggaaggcagat gctggaccac 420

ttccagggca caccccacca tggtgtctac tcccgagagg aggagctact gccggagcgc 480

aagcgcctgg gcgtcttcgg aatcacctct tatgacttcc acagtgagag cggcctttc 540

ctcttccagg ccagcaatag cctgtccac tgcagggatg gtggcaagaa tggctttatg	600
gtgtccccga tgaagccact ggagatcaag actcagtgtt ctgggccacg catggacccc	660
aaaatctgcc ctgcccaccc tgccttcttc tccttcatca ataacagcga cctgtgggtg	720
gccaacatcg agacaggcga ggagcggcgg ctgaccttct gccacccaagg tttatccaat	780
gtcctggatg accccaagtc tgcggtgtg gccacctcg tcatacagga agagttcgac	840
cgcttcactg ggtactggtg gtgccccaca gcctcctggg aaggttcaga gggcctcaag	900
acgctgcgaa tcctgtatga ggaagtcgat gagtccgagg tggaggtcat tcacgtcccc	960
tctcctgcgc tagaagaaaag gaagacggac tcgtatcggt acccccaggac aggcagcaag	1020
aatcccaaga ttgccttgaa actggctgag ttccagactg acagccaggg caagatcgtc	1080
tcgaccaggc agaaggagct ggtgcagccc ttcaagtcgc tgttcccggaa ggtggagtac	1140
atcgccaggg ccgggtggac ccgggatggc aaatacgcct gggccatgtt cctggacccgg	1200
ccccagcagt ggctccagct cgtcctctc ccccccggccc tgttcatccc gagcacagag	1260
aatgaggagc agcggctagc ctctgcaga gctgtcccca ggaatgtcca gccgtatgtg	1320
gtgtacgagg aggtcaccaa cgtctggate aatgttcatg acatcttcta tcccttcccc	1380
caatcagagg gagaggacga gctctgttt ctccgcgcga atgaatgcaa gacccggcttc	1440
tgccatttgt accaaagtca cgcgcgtttta aaatcccagg gtcacgattt gagtggagccc	1500
ttcagccccc gggaaagatga atttaagtgc cccattaagg aagagatggc tctgaccagc	1560
ggtgaatggg aggtttggc gaggcacggt tccaaagatct gggtaatga ggagaccaag	1620
ctgggtgtact tccagggcac caaggacacg cccgtggage accacctcta cgtggtcagc	1680
tatgaggcgg ccggcgagat cgtacgcctc accacgcggc gcttctccca tagtgcgtcc	1740
atgagccaga acttcgacat gttcgtcagc cactacagca gctgtgagcac gcccgcgtc	1800
gtgcacgtct acaagctgag cggcccccgcac gacgacccccc tgcacacaagca gccccgccttc	1860
tgggctagca tgatggaggc agccagctgc ccccccggatt atgttcctcc agagatcttc	1920
catttccaca cgcgcgtcgga tgcgcggctc tacggcatga tctacaagec ccacgccttg	1980
cagccagggc agaagcaccc caccgtccctc tttgtatatg gaggccccca ggtgcagctg	2040
gtgaataact cttcaaaagg catcaagtac ttgcggctca acacactggc ctccctggc	2100
tacgcccgtgg ttgtgattga cggcaggggc tcctgtcage gagggcttcg gttcgaaggg	2160
gcctgaaaa accaaatggg ccaggtggag atcgaggacc aggtggaggg cctgcagttc	2220
gtggccgaga agtatggctt cattgacttg agccgagtcg ccatccatgg ctggtcctac	2280
gggggcttcc tctcgctcat ggggctaatac cacaagcccc aggtgttcaa ggtggccatc	2340

gcgggtgccc cggtcaccgt ctggatggcc tacgacacag ggtacactga gcgctacatg 2400
gacgtccctg agaacaacca gcacggctat gaggcgggtt ccgtggctct gcacgtggag 2460
aagctgcccc aatgagccaa ccgcttgctt atcctccacg gtttctgga cgaaaacgtg 2520
cacttttcc acacaaaactt cctcgcttcc caactgatcc gagcaggaa accttaccag 2580
ctccagatct accccaacga gagacacagt attcgctgcc ccgagtcggg cgagcactat 2640
gaagtcacgt tgctgcactt tctacagggaa tacctc 2676

<210> 2

<211> 892

<212> PRT

<213> Homo sapiens

<400> 2

Met Arg Lys Val Lys Lys Leu Arg Leu Asp Lys Glu Asn Thr Gly Ser
1 5 10 15

Trp Arg Ser Phe Ser Leu Asn Ser Glu Gly Ala Glu Arg Met Ala Thr
20 25 30

Thr Gly Thr Pro Thr Ala Asp Arg Gly Asp Ala Ala Ala Thr Asp Asp
35 40 45

Pro Ala Ala Arg Phe Gln Val Gln Lys His Ser Trp Asp Gly Leu Arg
50 55 60

Ser Ile Ile His Gly Ser Arg Lys Tyr Ser Gly Leu Ile Val Asn Lys
65 70 75 80

Ala Pro His Asp Phe Gln Phe Val Gln Lys Thr Asp Glu Ser Gly Pro
85 90 95

His Ser His Arg Leu Tyr Tyr Leu Gly Met Pro Tyr Gly Ser Arg Glu
100 105 110

Asn Ser Leu Leu Tyr Ser Glu Ile Pro Lys Lys Val Arg Lys Glu Ala
115 120 125

Leu Leu Leu Leu Ser Trp Lys Gln Met Leu Asp His Phe Gln Ala Thr
130 135 140

Pro His His Gly Val Tyr Ser Arg Glu Glu Glu Leu Leu Arg Glu Arg
145 150 155 160

Lys Arg Leu Gly Val Phe Gly Ile Thr Ser Tyr Asp Phe His Ser Glu
165 170 175

Ser Gly Leu Phe Leu Phe Gln Ala Ser Asn Ser Leu Phe His Cys Arg
180 185 190

Asp Gly Gly Lys Asn Gly Phe Met Val Ser Pro Met Lys Pro Leu Glu
195 200 205

Ile Lys Thr Gln Cys Ser Gly Pro Arg Met Asp Pro Lys Ile Cys Pro
210 215 220

Ala Asp Pro Ala Phe Phe Ser Phe Ile Asn Asn Ser Asp Leu Trp Val
225 230 235 240

Ala Asn Ile Glu Thr Gly Glu Glu Arg Arg Leu Thr Phe Cys His Gln
245 250 255

Gly Leu Ser Asn Val Leu Asp Asp Pro Lys Ser Ala Gly Val Ala Thr
260 265 270

Phe Val Ile Gln Glu Glu Phe Asp Arg Phe Thr Gly Tyr Trp Trp Cys
275 280 285

Pro Thr Ala Ser Trp Glu Gly Ser Glu Gly Leu Lys Thr Leu Arg Ile
290 295 300

Leu Tyr Glu Glu Val Asp Glu Ser Glu Val Glu Val Ile His Val Pro
305 310 315 320

Ser Pro Ala Leu Glu Glu Arg Lys Thr Asp Ser Tyr Arg Tyr Pro Arg
325 330 335

Thr Gly Ser Lys Asn Pro Lys Ile Ala Leu Lys Leu Ala Glu Phe Gln
340 345 350

Thr Asp Ser Gln Gly Lys Ile Val Ser Thr Gln Glu Lys Glu Leu Val
355 360 365

Gln Pro Phe Ser Ser Leu Phe Pro Lys Val Glu Tyr Ile Ala Arg Ala
370 375 380

Gly Trp Thr Arg Asp Gly Lys Tyr Ala Trp Ala Met Phe Leu Asp Arg
385 390 395 400

Pro Gln Gln Trp Leu Gln Leu Val Leu Pro Pro Ala Leu Phe Ile
405 410 415

Pro Ser Thr Glu Asn Glu Glu Gln Arg Leu Ala Ser Ala Arg Ala Val
420 425 430

Pro Arg Asn Val Gln Pro Tyr Val Val Tyr Glu Glu Val Thr Asn Val
435 440 445

Trp Ile Asn Val His Asp Ile Phe Tyr Pro Phe Pro Gln Ser Glu Gly
450 455 460

Glu Asp Glu Leu Cys Phe Leu Arg Ala Asn Glu Cys Lys Thr Gly Phe
465 470 475 480

Cys His Leu Tyr Lys Val Thr Ala Val Leu Lys Ser Gln Gly Tyr Asp
485 490 495

Trp Ser Glu Pro Phe Ser Pro Gly Glu Asp Glu Phe Lys Cys Pro Ile
500 505 510

Lys Glu Glu Met Ala Leu Thr Ser Gly Glu Trp Glu Val Leu Ala Arg
515 520 525

His Gly Ser Lys Ile Trp Val Asn Glu Glu Thr Lys Leu Val Tyr Phe
530 535 540

Gln Gly Thr Lys Asp Thr Pro Leu Glu His His Leu Tyr Val Val Ser
545 550 555 560

Tyr Glu Ala Ala Gly Glu Ile Val Arg Leu Thr Thr Pro Gly Phe Ser
565 570 575

His Ser Cys Ser Met Ser Gln Asn Phe Asp Met Phe Val Ser His Tyr
580 585 590

Ser Ser Val Ser Thr Pro Pro Cys Val His Val Tyr Lys Leu Ser Gly
595 600 605

Pro Asp Asp Asp Pro Leu His Lys Gln Pro Arg Phe Trp Ala Ser Met
610 615 620

Met Glu Ala Ala Ser Cys Pro Pro Asp Tyr Val Pro Pro Glu Ile Phe
625 630 635 640

His Phe His Thr Arg Ser Asp Val Arg Leu Tyr Gly Met Ile Tyr Lys
645 650 655

Pro His Ala Leu Gln Pro Gly Lys Lys His Pro Thr Val Leu Phe Val
660 665 670

Tyr Gly Gly Pro Gln Val Gln Leu Val Asn Asn Ser Phe Lys Gly Ile
675 680 685

Lys Tyr Leu Arg Leu Asn Thr Leu Ala Ser Leu Gly Tyr Ala Val Val
690 695 700

Val Ile Asp Gly Arg Gly Ser Cys Gln Arg Gly Leu Arg Phe Glu Gly
705 710 715 720

Ala Leu Lys Asn Gln Met Gly Gln Val Glu Ile Glu Asp Gln Val Glu
725 730 735

Gly Leu Gln Phe Val Ala Glu Lys Tyr Gly Phe Ile Asp Leu Ser Arg
740 745 750

Val Ala Ile His Gly Trp Ser Tyr Gly Gly Phe Leu Ser Leu Met Gly
755 760 765

Leu Ile His Lys Pro Gln Val Phe Lys Val Ala Ile Ala Gly Ala Pro
770 775 780

Val Thr Val Trp Met Ala Tyr Asp Thr Gly Tyr Thr Glu Arg Tyr Met
785 790 795 800

Asp Val Pro Glu Asn Asn Gln His Gly Tyr Glu Ala Gly Ser Val Ala
805 810 815

Leu His Val Glu Lys Leu Pro Asn Glu Pro Asn Arg Leu Leu Ile Leu
820 825 830

His Gly Phe Leu Asp Glu Asn Val His Phe Phe His Thr Asn Phe Leu
835 840 845

Val Ser Gln Leu Ile Arg Ala Gly Lys Pro Tyr Gln Leu Gln Ile Tyr
850 855 860

Pro Asn Glu Arg His Ser Ile Arg Cys Pro Glu Ser Gly Glu His Tyr
865 870 875 880

Glu Val Thr Leu Leu His Phe Leu Gln Glu Tyr Leu
885 890

<210> 3

<211> 882

<212> PRT

<213> Homo sapiens

<400> 3

Met Ala Ala Ala Met Glu Thr Glu Gln Leu Gly Val Glu Ile Phe Glu
1 5 10 15

Thr Ala Asp Cys Glu Glu Asn Ile Glu Ser Gln Asp Arg Pro Lys Leu
20 25 30

Glu Pro Phe Tyr Val Glu Arg Tyr Ser Trp Ser Gln Leu Lys Lys Leu
35 40 45

Leu Ala Asp Thr Arg Lys Tyr His Gly Tyr Met Met Ala Lys Ala Pro
50 55 60

His Asp Phe Met Phe Val Lys Arg Asn Asp Pro Asp Gly Pro His Ser
65 70 75 80

Asp Arg Ile Tyr Tyr Leu Ala Met Ser Gly Glu Asn Arg Glu Asn Thr
85 90 95

Leu Phe Tyr Ser Glu Ile Pro Lys Thr Ile Asn Arg Ala Ala Val Leu
100 105 110

Met Leu Ser Trp Lys Pro Leu Leu Asp Leu Phe Gln Ala Thr Leu Asp
115 120 125

Tyr Gly Met Tyr Ser Arg Glu Glu Glu Leu Leu Arg Glu Arg Lys Arg
130 135 140 140

Ile Gly Thr Val Gly Ile Ala Ser Tyr Asp Tyr His Gln Gly Ser Gly
145 150 155 160

Thr Phe Leu Phe Gln Ala Gly Ser Gly Ile Tyr His Val Lys Asp Gly
165 170 175

Gly Pro Gln Gly Phe Thr Gln Gln Pro Leu Arg Pro Asn Leu Val Glu
180 185 190

Thr Ser Cys Pro Asn Ile Arg Met Asp Pro Lys Leu Cys Pro Ala Asp
195 200 205

Pro Asp Trp Ile Ala Phe Ile His Ser Asn Asp Ile Trp Ile Ser Asn
210 215 220

Ile Val Thr Arg Glu Glu Arg Arg Leu Thr Tyr Val His Asn Glu Leu
225 230 235 240

Ala Asn Met Glu Glu Asp Ala Arg Ser Ala Gly Val Ala Thr Phe Val
245 250 255

Leu Gln Glu Glu Phe Asp Arg Tyr Ser Gly Tyr Trp Trp Cys Pro Lys
260 265 270

Ala Glu Thr Thr Pro Ser Gly Gly Lys Ile Leu Arg Ile Leu Tyr Glu
275 280 285

Glu Asn Asp Glu Ser Glu Val Glu Ile Ile His Val Thr Ser Pro Met
290 295 300

Leu Glu Thr Arg Arg Ala Asp Ser Phe Arg Tyr Pro Lys Thr Gly Thr
305 310 315 320

Ala Asn Pro Lys Val Thr Phe Lys Met Ser Glu Ile Met Ile Asp Ala
325 330 335

Glu Gly Arg Ile Ile Asp Val Ile Asp Lys Glu Leu Ile Gln Pro Phe
340 345 350

Glu Ile Leu Phe Glu Gly Val Glu Tyr Ile Ala Arg Ala Gly Trp Thr
355 360 365

Pro Glu Gly Lys Tyr Ala Trp Ser Ile Leu Leu Asp Arg Ser Gln Thr
370 375 380

Arg Leu Gln Ile Val Leu Ile Ser Pro Glu Leu Phe Ile Pro Val Glu
385 390 395 400

Asp Asp Val Met Glu Arg Gln Arg Leu Ile Glu Ser Val Pro Asp Ser
405 410 415

Val Thr Pro Leu Ile Ile Tyr Glu Glu Thr Thr Asp Ile Trp Ile Asn
420 425 430

Ile His Asp Ile Phe His Val Phe Pro Gln Ser His Glu Glu Glu Ile
435 440 445

Glu Phe Ile Phe Ala Ser Glu Cys Lys Thr Gly Phe Arg His Leu Tyr
450 455 460

Lys Ile Thr Ser Ile Leu Lys Glu Ser Lys Tyr Lys Arg Ser Ser Gly
465 470 475 480

Gly Leu Pro Ala Pro Ser Asp Phe Lys Cys Pro Ile Lys Glu Glu Ile
485 490 495

Ala Ile Thr Ser Gly Glu Trp Glu Val Leu Gly Arg His Gly Ser Asn
500 505 510

Ile Gln Val Asp Glu Val Arg Arg Leu Val Tyr Phe Glu Gly Thr Lys
515 520 525

Asp Ser Pro Leu Glu His His Leu Tyr Val Val Ser Tyr Val Asn Pro
530 535 540

Gly Glu Val Thr Arg Leu Thr Asp Arg Gly Tyr Ser His Ser Cys Cys
545 550 555 560

Ile Ser Gln His Cys Asp Phe Phe Ile Ser Lys Tyr Ser Asn Gln Lys
565 570 575

Asn Pro His Cys Val Ser Leu Tyr Lys Leu Ser Ser Pro Glu Asp Asp
580 585 590

Pro Thr Cys Lys Thr Lys Glu Phe Trp Ala Thr Ile Leu Asp Ser Ala
595 600 605

Gly Pro Leu Pro Asp Tyr Thr Pro Pro Glu Ile Phe Ser Phe Glu Ser
610 615 620

Thr Thr Gly Phe Thr Leu Tyr Gly Met Leu Tyr Lys Pro His Asp Leu
625 630 635 640

Gln Pro Gly Lys Lys Tyr Pro Thr Val Leu Phe Ile Tyr Gly Pro
645 650 655

Gln Val Gln Leu Val Asn Asn Arg Phe Lys Gly Val Lys Tyr Phe Arg
660 665 670

Leu Asn Thr Leu Ala Ser Leu Gly Tyr Val Val Val Val Ile Asp Asn
675 680 685

Arg Gly Ser Cys His Arg Gly Leu Lys Phe Glu Gly Ala Phe Lys Tyr
690 695 700

Lys Met Gly Gln Ile Glu Ile Asp Asp Gln Val Glu Gly Leu Gln Tyr
705 710 715 720

Leu Ala Ser Arg Tyr Asp Phe Ile Asp Leu Asp Arg Val Gly Ile His
725 730 735

Gly Trp Ser Tyr Gly Gly Tyr Leu Ser Leu Met Ala Leu Met Gln Arg
740 745 750

Ser Asp Ile Phe Arg Val Ala Ile Ala Gly Ala Pro Val Thr Leu Trp
755 760 765

Ile Phe Tyr Asp Thr Gly Tyr Thr Glu Arg Tyr Met Gly His Pro Asp
770 775 780

Gln Asn Glu Gln Gly Tyr Tyr Leu Gly Ser Val Ala Met Gln Ala Glu
785 790 795 800

Lys Phe Pro Ser Glu Pro Asn Arg Leu Leu Leu Leu His Gly Phe Leu
805 810 815

Asp Glu Asn Val His Phe Ala His Thr Ser Ile Leu Leu Ser Phe Leu
820 825 830

Val Arg Ala Gly Lys Pro Tyr Asp Leu Gln Ile Tyr Pro Gln Glu Arg
835 840 845

His Ser Ile Arg Val Pro Glu Ser Gly Glu His Tyr Glu Leu His Leu
850 855 860

Leu His Tyr Leu Gln Glu Asn Leu Gly Ser Arg Ile Ala Ala Leu Lys
865 870 875 880

Val Ile

<210> 4

<211> 611

<212> DNA

<213> *Homö sapiens*

<400> 4

gagggttcaga gggctcaaga cgctgcgaat cctgtatgag gaagtgcgtg agtccgaggt 60

ggaggttcatt cacgtccccct ctccctgcgtc agaagaaaagg aagacggact cgtatcggt 120

ccccaggaca ggcagcaaga atcccaagat tgccctgaaa ctggctgagt tccagactga 180

cagccagggc aagatcgtct cgacccagga gaaggagctg gtgcagccct tcagctcgct 240

gttcccgaag gtggagtaca tcgcccaggc cgggtggacc cgggatggca aatacgctg 300

ggccatgttc ctggaccggc cccagcagtg gctccagctc gtcctctcc ccccgccct 360

gttcatcccc agcacagaga atgaggagca gcggctagcc tctgccagag ctgtccccag 420

gaatgtccag ccgtatgtgg tgtacgagga ggtcaccaac gtctggatca atgttcatga 480

catcttctat cccttccccc aatcagaggg agaggacgag ctctgcttcc tccgcgccaa 540

tgaatgcaag accggcttct gccatttcta caaagtcaacc gccgtttaa aatccagggc 600

tacgatttggaa g 611

<210> 5
<211> 1080
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (1079)..(1080)
<223> N=a,t,g or c

<400> 5
ggggagggttt tggcgaggca cggatccaag ggcaccaagg acacgcccgt ggagcaccac 60
ctctacgtgg tcagctatga ggcggccggc gagatcgtac gcctcaccac gccccggcttc 120
tccccatagct gctccatgag ccagaacttc gacatgttcg tcagccacta cagcagcgtg 180
agcacgcccgc cctgcgtgca cgtctacaag ctgagcggcc ccgacgacga cccccctgcac 240
aagcagcccc gtttctgggc tagcatgatg gaggcagcca gctgcccccc ggattatgtt 300
cctccagaga tcttccatTT ccacacgcgc tcggatgtgc ggctctacgg catgatctac 360
aagccccacg ctttgcagcc agggagaag cacccccaccg tcttctttgt atatggaggc 420
ccccaggtgc agctggtgaa taactcccttc aaaggcatca agtacttgcg gctcaacaca 480
ctggccctccc tgggctacgc cgtgggtgtg attgacggca gggctctgt cagcagggc 540
ttcggttcga agggggccctg aaaaaacaaa tgggccaggt ggagatcgag gaccaggtgg 600
gagggcctgc agttccgtga gcgagaagta tggcttcata cgaactgagc cgagttgcc 660
tccaatggct gggctacgg ggacttactc tcgctcatgg gggtaatcca caagccccagg 720
gggtcagggg caatcgcgag tgccccggca cggctggatg gccaagaaac agcgtacact 780
gagcggaaagg gacggccccgg aaaaccagac aggaaaaggg gagtccgggg cccgcgtcagg 840
gagaaagtgc cacgaagcca cagcggggat cacacagagg cgtgcggaca cgggggggtt 900
ccacaaaaaca cgacacacaagg agacggaaa caccacaac aataccagga acaatgaggc 960
gcggggaaac gacacgagaa caacacatgg acgcgacgaa caaagcaatg cgacagcaga 1020
gacgcgggac gaccaggacg ccagttcgca cggctgcgcgg agcgaccagn 1080

<210> 6

<211> 744

<212> DNA

<213> Homo sapiens

<400> 6

gctcgatgt gcggtctac ggcatgatct acaagccccca cgccttgcag ccagagaaga	60
agcaccccac cgtcctttt gtatatggag gcccccaaggt gcagctggtg aataactcct	120
tcaaaggcat caagtacttg cggctcaaca cactggcctc cctggctac gccgtggttg	180
tgattgacgg caggggctcc tgcagcgag ggttcgggtt cgaaggggcc ctgaaaaacc	240
aaatgggcca ggtggagatc gaggaccagg tggagggct gcagttcgtg gccgagaagt	300
atggcttcat cgacactgag ccgagttgcc atccatggct ggtcctacgg gggatccctc	360
tcgctcatgg ggctaattca caagccccag gtgttcaagg tggccatcgc ggggtccccgg	420
gtcacccgtct ggatggctta cgacacaggg tacactgagc gctacatgga cgtccctgag	480
aacaaccagg acggctatga ggccgggtcc gtggccctgc acgtggagaa gctgcccatt	540
gagcccaacg attgcttatac ctccacgggtt cctggacgaa aacgtgcact ttttccacac	600
aaattcctcg tctcccaact gatccgagca gggaaacctt acagcgtcgg atcaacccaa	660
gagagaacag ttctcgatgcc gagtcggcga catatgagtc gttgatgact tctaaggaaa	720
ctctgacctg cacggacgcc atta	744

<210> 7

<211> 506

<212> DNA

<213> Homo sapiens

<400> 7

aaagcagagc tcgtctctc cctctgattt gggaaaggga tagaagatgt catgaacatt	60
gatccagacg ttgggtgaccc cctcgtacac cacatacggc tggacattcc tggggacagc	120
tctggcagag gctagccgct gctcttcatt ctctgtgctc gggatgaaca gggccgggg	180
gaggaggacg agctggagcc actgctgggg ccggtccagg aacatggccc aggcttattt	240
gccatccccgg gtccacccgg ccctggcgat gtactccacc ttccggaaaca gcgagctgaa	300

gggctgcacc agctccttct cctgggtcga gacgatcttgc cctggctgt cagtctggaa 360
ctcagccagt ttcaaggcaa tcttgggatt cttgtgcct gtcctgggtt accgatacga 420
gtccgttcc ctttcttcta ggcaggaga gggacgtga atgacctcca cctcggactc 480
atcgacttcc tcatacagga ttgcga 506

<210> 8

<211> 753

<212> DNA

<213> Homo sapiens

<220>

<221> misc_feature

<222> (688) .. (689)

<223> n=a,t,g or c

<400> 8
attacatcta ttttaaagga aagcaaatat aaacgatcca gtgggtggct gcctgctcca 60
agtgatttca agtgtcctat caaagaggag atagcaatta ccagtggtga atgggaagtt 120
cttggccggc atggatctaa tatccaagtt gatgaagtca gaaggctggat atatttgaa 180
ggcacccaaag actccccctt agagcatcac ctgtacgtac tcagttacgt aaatcctgg 240
gaggtgacaa ggctgactga ccgtggctac tcacattttt gctgcacatcag tcagcactgt 300
gacttcttta taagtaagta tagtaaccag aagaatccac actgtgtgtc ctttacaag 360
ctatcaagtc ctgaagatga cccaaacttgc aaaacaaagg aattttggc caccatttt 420
gattcagcag gtccttttcc tgactatact cttccagaaa ttttctttt tgaaagtact 480
actggatttta cattgtatgg gatgtctac aagcctcatg atctacagcc tggaaagaaa 540
tatacctactg tgctgttcat atatgggtgt ctcaggtgc agttgggtgaa taatcggttt 600
aaaggagtca agtatttccg cttgaataacc ctgccttc taggttatgt gggtttagtg 660
atagacaaca ggggatcctg tcaccgangg cttaaatttg aaggccctt taaatataaa 720
atgggtcaaa tagaaattga cgatcaggtg gga 753

<210> 9

<211> 494

<212> DNA

<213> Homo sapiens

<400> 9

aaagcagagc tcgtcctctc cctctgattt gggaaaggga tagaagatgt catgaacatt 60
gatccagacg ttggtgaccc cctctgtacac cacatacggc tggacatcc tggggacagc 120
tctggcagag gctagccgct gtccttcatt ctctgtgctc gggatgaaca gggccggggg 180
gaggaggacg agctggagcc actgctgggg ccggtccagg aacatggccc aggcttattt 240
gccatccccgg gtccacccgg ccctggcgat gtactccacc ttcgggaaca gcgagctgaa 300
gggatgcacc agtccttctc cctgggtcga gacgatcttgc ccctggctgt cagtctggaa 360
ctcagccagt ttcaaggcaa tcttggata cttgtgcct gtcctgggtt accgatacga 420
gtccgttctc ctttcttcta ggcgaggaga ggggacgtga atgaccttcca cctcggactc 480
atcgacttcc tcat 494

<210> 10

<211> 754

<212> DNA

<213> Homo sapiens

<400> 10

cggctccaag ggcaccaagg acacgcccgtt ggagcaccac ctctacgtgg tcagctatga 60
ggcggccggc gagatcgtaa gcctcaccac gcccggcttc tcccatagct gtcctatgag 120
ccagaacttc gacatgttcg tcagccacta cagcagcgtg agcacgcccgc cctgcgtgca 180
cgtctacaag ctgagcggcc cccgacgacga cccctgcac aagcagcccc gtttctggc 240
tagcatgatg gaggcagcca gtcgtccccgg ggattatgtt cttccagaga tcttccattt 300
ccacacgcgc tggatgtgc ggctctacgg catgatctac aagccccacg cttgcagcc 360
agggaaagaag caccccacgg tcctctttt gatatggagg ccccccagggtg cagctgggtga 420
ataactcctt caaaggcatc aagtacttgc ggctcaacac actggcctcc ctgtggctac 480
ggcgtggttt gtgattgacg gcaggggctc ctgtcagcga gggcttcgggt tcgaaggggc 540

ctgaaaaacc aaatgggcca gtgtggagat cgaggacctg gtggagggcc tgcagtgcgt	600
ggccgagaag tatggcttct cgactgagcc gagttgcctc cttggctgtc ctacggggcc	660
ttctctcgct catggggcta tccacagccc gtgtccgtgg ctgcgggtgc cggcccttgg	720
ttgctacgac cggtcctggg ctctgatctg gaac	754

<210> 11

<211> 1169

<212> DNA

<213> Homo sapiens

<400> 11	
ggggctgtcg tccccgctc cggccacttc cggggtcgca gtccccggca tggagcccg	60
accgtgaggc gcccgtggac cggggacgac ctggccagtc cggccgcgc cccacgtccc	120
ggtctgtgtc ccacgcctgc agctggaatg gaggtctctt ggacccttta gaaggcacc	180
ctgcccctctt gaggtcagct gagcggttaa tgccggaaatgt taagaaaactg cgcctggaca	240
aggagaacac cggaaagtgg agaagcttct cgctgaattt cggggggct gagaggatgg	300
ccaccacccgg gaccccaacg gcccggcgg ggcacgcgc cggccacagat gacccggccg	360
cccgcttcca ggtgcagaag cactcgtggg acgggctccg gagcatcatc cacggcagcc	420
gcaagtactc gggccttatt gtcaacaagg cggccacacg cttccagttt gtgcagaaga	480
cgatgagtc tgggccccac tcccacccgc tctactaccc gggaatgcca tatggcagcc	540
gagagaactc cctcccttac tctgagattt ccaagaaggt cggaaaagag gctctgtgc	600
tccctgtcct ggaagcagat gctggcatca ttccaggcc acggccacaca tgggggtcta	660
ctctcggag gaggagctgc tgagggagcg gaaacgcctg ggggcttcc ggcacatcac	720
cctacgactt ccacagcgag agtggccctt tcccttcca gcccacacag cttctccac	780
tggcgccacg gcggaagaac ggctcatggg tgccctatg aaccgtggga attcacaccc	840
aaggacagg gccccgagtg ggagcgaaaa tctgccccgc gaccatgacg tatatggcgt	900
caaacaacaa caggcacccg tgggtgtgcc catcctaaca cgggacgaaa cgagcggacc	960
ctccgcacaa gggatcacgc cgcacagacc caactcagtg gggggacact gcagagacaa	1020
ccaacacgaa tcgtacacgt ggccaccc cgcacacag aagcaacaca gcaacgaaga	1080
agcagacaca gggcgcaagc cgacctagac agagcagacc gcaggacggt acgagcacaa	1140
acatctgaag accgcagcca cggcccgcc	1169

<210> 12

<211> 1002

<212> DNA

<213> Homo sapiens

<400> 12

gagtcaagta	tttcccgtt	gaatacccta	gcctctctag	gttatgtgggt	tgttagtgata	60
gacaacaggg	gatctctgtca	ccgagggctt	aaatttgaag	gcgcctttaa	atataaaatg	120
ggtaaaatag	aaatttgacga	tcaggtggaa	ggactccaat	atctagcttc	tcgatatgat	180
ttcattgact	tagatcgtgt	gggcattccac	ggctggtcct	atggaggata	cctctccctg	240
atggcattaa	tgcagaggc	agatatcttc	agggttgcta	ttgctgggc	cccagtca	300
ctgtggatct	tctatgatac	aggatacacg	gaacgttata	tgggtcaccc	tgaccagaat	360
gaacagggct	attacttagg	atctgtggcc	atgcaagcag	aaaagttccc	ctctgaacca	420
aatcgtttac	tgctcttaca	tggtttcctg	gatgagaatg	tccatttgc	acataccagt	480
atattactga	gttttttagt	gagggctgga	aagccatatg	atttacagat	ctatcctcag	540
gagagacaca	gcataagagt	tcctgaatcg	ggagaacatt	atgaactgca	tctttgcac	600
taccttcaag	aaaaccttgg	atcacgtatt	gctgctctaa	aagtgtatata	attttgacct	660
gtgtagaact	ctctggata	cactggatt	tgaccaaatt	aggacgtgta	atcgaaagcga	720
aaacccagaa	atggtcatcg	ccattgtgtt	acctgcattt	ttagcattta	cttctggaaa	780
attaatgttg	gtgccatgca	ggcgcttacg	ggtggggaa	attaatactt	taacccatg	840
tgctaata	tgtatttctc	ggaaaccagt	cctagatttc	aaaaaaaaa	accgagattt	900
tttccagagg	gwgcaagggg	gccacaggc	ttaaaaggct	ggaagagacc	gtcaatgtgc	960
cagtgtgcaa	actcttttgt	gagagaattt	tatgataggg	ag		1002

<210> 13

<211> 969

<212> DNA

<213> Homo sapiens

<220>

<221> misc_feature

<222> (400)..(401)

<223> n=a,t,g or c

<400> 13

ggcttctgcc	atgtgtacaa	agtacccgcc	gttttaaaat	cccagggcta	cgatgggagt	60
gagcccttca	ccccccccca	agatgaattt	aagtgcggca	ttaaggaaga	gatggctctg	120
accagcggtg	aatgggaggt	tttggcgagg	cacgggttcca	agatctgggt	caatgaggag	180
accaagctgg	tgtacttcca	gggcaccaag	gacacgcccgc	tggagcacca	cctctacgtg	240
gtcagctatg	aggcgcccg	cgagatcgta	cgcctcaacc	aggcccggtt	tctccatag	300
ctggctccat	gagccagaac	ttcgacatgt	tcgtcagcca	ctacagcagc	gtgagcacgc	360
cgccctgcgt	gcacgtctaa	caagctggag	ctggcccg	ngaggaaccc	ctgcacaagc	420
agcccggtt	ctgggcttagc	atgatggagg	cagccaggtg	ccccggggat	tatgttcctc	480
cagagatctt	ccatttccac	acgcgcgtcg	atgtcggtc	tacggcatga	tctagaagcc	540
caagcttgg	gccaggacga	agaccaacgg	cctcttggat	ttggggccca	ggtgccgtgg	600
ggctaattcct	tcaagggctc	aggacatgcg	ggtacacaat	gggctctgg	gtacgcccgg	660
aaggagatgc	ggagggggcc	ggacaggagg	ggcaggc	agggccgaga	aaaattggc	720
cgggaaccgg	acctggagag	gcaaaattgg	cacaacagt	gttaaagcga	gaaagggaca	780
ccaagcgagg	gacaggagga	cctacaagt	ggtgacaacc	cgcgtagaga	gagaagacgg	840
acccacgg	tgcaaagaaa	cacgagaacc	aacaaatcaa	aatgtagcgag	cgcacgaaac	900
aatgcaagcg	taagttcttc	ttcgctgccc	tcgtcgcgc	cgtctgtct	gctgttgg	960
ggagaggag						969

<210> 14

<211> 442

<212> DNA

<213> Homo sapiens

<400> 14
tggcgaattc ggcacgaggg ggagggttttgcgaggcacg gctccaaggg caccaggac 60
acgcccgtgg agcaccaccc ttagtggc agctatgagg cggccggcga gatcgtaacgc 120
ctcaccacgc cccggcttctc ccatagctgc tccatgagcc agaacttcga catttcgac 180
agccactaca gcagcgtgag cacggccccc tgcgtgcacg tctacaagct gagcggcccc 240
gacgacgacc ccctgcacaa gcagccccgc ttctgggcta gcatgatgga ggcagccagc 300
tgccccccgg attatgttcc tccagagatc ttccatttcc acacgcgcgc ggtatgtgcgg 360
ctctacggca ttagtctacaa gccccacgccc ttgcagccag ggaagaagca ccccacccgtc 420
ctctttgtat atggaggccc cc 442

<210> 15

<211> 735

<212> DNA

<213> Homo sapiens

<220>

<221> misc_feature

<222> (708) .. (709)

<223> n=a,t,g or c

<400> 15
caacaggggca tcctgtcacc gagggcttaa atttgaaggc gcctttaaat ataaaatggg 60
tcaaataagaa attgacgatc aggtggaaagg actccaaat attagtttcc gatatgattt 120
cattgactta gatcgtgtgg gcatccacgg ctggcctat ggaggatacc tctccctgat 180
ggcattaaatg cagaggtcag atatcttcag ggttgctatt gctggggccc cagtcactct 240
gtggatcttc tatgatacag gatacacgga acgttatatg ggtcaccctg accagaatga 300
acagggttat tacttaggat ctgtggccat gcaagcagaa aagttccctt ctgaacccaaa 360
tcgtttactg ctcttacatg gtttcctggta tgagaatgtc cattttgcac ataccagtat 420
attactgagt ttttttagtga gggctggaaa gccatatgtt ttacagatct atcctcagga 480
gagacacacgc ataaagagtcc ctgaatcggg agaacattat gaaactgcac ttttgcacta 540
ccttcaagaa aaccttggat cacgtattgc tgctctaaaa gtgatataat tttgacctgt 600

gtagaactct ctggtataca ctggctattt aaccaaatga ggaggtttaa tcaacagaaa 660
acacagaatt gatcatcaca ttttgatacc tgccatgtaa catctactnc tgaaaataaa 720
tgtggtgcca tgcag 735

<210> 16

<211> 888

<212> DNA

<213> Homo sapiens

<400> 16
gtctacgggg gcttctctc gtcatgggg ctaatccaca agccccaggt gttcaaggtg 60
gccatcgccc gtcccccggt caccgtctgg atggcctacg acacagggtt cactgagcgc 120
tacatggacg tccctgagaa caaccagcac ggctatgagg cgggttccgt ggctctgcac 180
gtggagaagc tgcccaatga gccccacccgc ttgcttatcc tccacggctt cctggacgaa 240
aacgtgcact ttttccacac aaacttctc gtctcccaac tgatccgagc agggaaacct 300
taccagctcc agatctaccc caacgagaga cacagtattc gctgccccga gtcggggcag 360
caactatgaag tcaacgttgtc gcaacttcttca caggaataacc tctgagctg cccacccgg 420
gcccgcacat cacagcacaa gtggctgcag cctccgcggg gaaccaggcg ggagggactg 480
agtggccgc gggccccagt gaggcactt gtcccgccca gcgctggcca gccccgagga 540
gcccgccttc tcacccgcgg acgcctttta tccttttta aacgctcttgc ggttttatgt 600
ccgctgtttc ttgggttgcgg agacagagag atgggtgtc cggggccagcc ccttctctc 660
ccccgccttc gggaggagga ggtcacacgc tgatggcgc tggagaggca gaagagactc 720
agaggggggg gctgtttccg ctggggctcc tggacacttc tcaagtcccc tggccggca 780
ggccggtccc aggccccaaagc tgctttgtcg gtccccccggg cggttcccac ttgtgttggg 840
ttgggtggggg ggatttccat tttaaagagg gggggggcgc ctgttccc 888

<210> 17

<211> 454

<212> DNA

<213> Homo sapiens

<220>

<221> misc_feature

<222> (435) .. (436)

<223> n=a,t,g or c

<400> 17

tgccccacag	cctcctggga	aggttcagag	ggcctaaga	cgctgcgaat	cctgtatgag	60
gaagtcgatg	agtccgaggt	ggaggtcatt	cacgtccccct	ctcctgcgtct	agaagaaaagg	120
aagacggact	cgcatcggtt	ccccaggaca	ggcagcaaga	atcccaagat	tgccttgaaa	180
ctggctgagt	tccagactga	cagccagggc	aagatcgctt	cgaccccaga	gaaagagctg	240
gtgcagccct	tcagtcgct	gttcccgaag	gtggagtaca	tcgcccaggc	cgggtggacc	300
cgggatggca	aatacgcctg	ggccatgttc	ctggaccggc	cccagcagtg	gtccagctc	360
gtccttcctc	ccccggccct	gttcatcccc	agcacagaga	atgaggagca	gcggctagcc	420
tctggcgagc	tgtcncagga	atgtccagcc	gtat			454

<210> 18

<211> 452

<212> DNA

<213> Homo sapiens

<400> 18

gtcttaccc	gactttgtc	ttttggggtc	tttgctttt	tggggctttt	gtctatggtg	60
tccccatga	aaccgctgga	aatcaagacc	cagtgtcag	ggccccggat	ggaccccaaa	120
atctgcctg	ccgaccctgc	tttcttctcc	ttaatcaata	acagcgacct	gtgggtggcc	180
aacatcgaga	caggcgagga	gcggcggctg	accttctgcc	accaaggttt	atccaatgtc	240
ctggatgacc	ccaaagtctgc	gggtgtggcc	actttcgtca	tacaggaaga	gttcgaccgc	300
ttcaactgggt	actggtggtg	ccccacagcc	tcctggaaag	gttcagaggg	cctcaagacg	360
ctgcgaatcc	tgtatgagga	agtcgatgag	tccgaggtgg	aggtaattca	cgtccactct	420
cctgcgttag	aagaaaggaa	gacggactcg	ta			452

<210> 19

<211> 555

<212> DNA

<213> Homo sapiens

<400> 19

cgtcgtgtaa tccgtacaat gaacaaacag catattgaac cacacgtgac taacgcgtat	60
agtgcacagc attcgtcagg ctctgtcac ctccccggg gctgaagggc tcactccat	120
cgtagccctg ggattttaaa acggcggtga ctttgtacaa atggcagaag ccggcttgc	180
aattcattgg cgccggagaaaa gcagagctcg tcctctccct ctgattgggg gaaggatag	240
aagatgtcat gaacattgtat ccagacgttg gtgacctcct cgtacaccac atacggctgg	300
acattcctgg ggacagctct ggcagaggct agccgctgct cctcatttctc tgtgctcggg	360
atgaacaggg ccggggggag gaggacgagc tggagccact gctggggccg gtccaggaac	420
atggcccagg cgtatggcc atccgggtc cacccggccc tggcgatgtat cttcaccttc	480
gggaacacgcg agctgaaggg ctgcaccagc tccttcctt gggcgagac gatcttgcgg	540
tggctgtcag tctgg	555

<210> 20

<211> 343

<212> DNA

<213> Homo sapiens

<400> 20

gatcttgcgg tggctgtcag tctggactc agccagtttc aaggcaatct tggattctt	60
gctgcctgtc ctggggtacc gatacgagtc cgttttcctt tcttctagcg caggagaggg	120
gacgtaatg acctccaccc tggactcatac gacttcctca tacaggattc gcagcgtctt	180
gaggccctctt gAACCTCCC aggaggctgt gggcaccac cagtacccag tgaagcggtc	240
gaactcttcc tttatgacga aggtggccac acccgacagac ttggggtcat ccaggacatt	300
ggataaacct tggtggcaga aggtcagccg ccgttcctcg cct	343

<210> 21

<211> 317

<212> DNA

<213> Homo sapiens

<400> 21

aacgggtgat	gccccacgac	ttccagtttg	tgcagaagac	ggatgagtct	ggccccact	60
gaagcttctc	gctgaattcc	gagggggctg	agaggatggc	caccaccggg	accccaacgg	120
ccgaccgagg	cgacgcagcc	gccacagatg	acccggccgc	ccgcttccag	gtgcagaagc	180
actcggtgg	cgggctccgg	agcatcatcc	acggcagccg	caagtactcg	ggcctcattg	240
tcaacaaggc	gccccacgac	ttccagtttg	tgcagaagac	ggatgagtct	ggccccact	300
ccaaccgcat	cacgggt					317

<210> 22

<211> 520

<212> DNA

<213> Homo sapiens

<400> 22

ttaatgcgga	aggtaagaa	actgcgcctg	gacaaggaga	acaccggaag	ttggagaagc	60
ttctcgctga	attccgaggg	ggctgagagg	atggccacca	ccgggacccc	aacggccgac	120
cgaggcgcac	cagccgcccac	agatgacccg	gccgcggct	tccaggtgca	gaagcactcg	180
tgggacgggc	tccggagcat	catccacggc	agccgcaagt	actcgggcct	cattgtcaac	240
atcgagacag	gctggaggagcg	ggggctgacc	ttctgccacc	aaggtttatac	caatgtcctg	300
gatgacccca	agtctgcggg	tgtggccacc	ttcgtcatac	aggaagagtt	cgaccgcttc	360
actgggtact	ggtggtgccc	cacagcetcc	tgggaaggtt	cagagggcct	caagacgctg	420
cgaatcctgt	atgaggaagt	cgatgagtc	gaggtggagg	tcattcacgt	accctctct	480
gcgcgttagaag	aaaggaagac	ggactcgat	cggtacccca			

<210> 23

<211> 411

<212> DNA

<213> Homo sapiens

<400> 23

aggctgtggg gcaccaccag tacccagtga agcggtcgaa ctcttcctgt atgacgaacg	60
tggccacacc cgcagacttg gggtcatcca ggacatttggaa taaaccttgg tggcagaagg	120
tcagccgccc ctccctcgccct gtctcgcatg ttggccacccc acaggtcgct gttatttgatg	180
aaggagaaga aggcagggtc ggcagggcag attttgggtt ccatccgggg ccctgagcac	240
tgggtcttga tttccagcgg tttcataggg gacaccatga agccgttctt gccgcccgtcg	300
cggcagtgga agaggctgtt gctggctgg aagaggaagt ggccactctc gctgtggaag	360
tcgttaggagg tgatgccgaa gaccccccagg cgttcccgct ccctcagcag c	411

<210> 24

<211> 600

<212> DNA

<213> Homo sapiens

<400> 24

ttgtggctca gtgccaataa aactttatgtt atgaacacag gtgggggcc ggatttgacc	60
tgtggccgt aggtggccaa cccctggttt tgctaaggcc ctggcacagg ggaggtgctc	120
agtaaatctg ctgcctgaat aagccaaacag ttgggtgctc taagccctgc cagatcatgc	180
atgcactggg gaaggctggc ttcctggca tccctgttcc ttctccgca gggtgtgctg	240
gctgagtggg gggccgacc aatgaacaaa cagcatattt aaccacacgt gactaacgcg	300
atgagtcgac agcattcgac aggtctgtt cacctttccc ggggtgttgg ggctcaactcc	360
aatcgtagcc ctgggatttt aaaacggcgg tgactttgtt caaatggcag aagccggct	420
tgcattcatt ggcggggaga aagcagagct cgtccctctcc ctctgattgg gggaaaggat	480
agaagatgtc atgaacattt atccagacgt tggtgacctc ctccgtacac acatacggct	540
cggacattct gggacagct ctcgcagagg ctgcggccct gctctcatcc tctgtgtcg	600

<210> 25

<211> 645

<212> DNA

<213> Homo sapiens

<220>

<221> misc_feature

<222> (327) .. (328)

<223> n=a,t,g or c

<220>

<221> misc_feature

<222> (349) .. (350)

<223> n=a,t,g or c

<400> 25

agatcgagga ccaggtggag ggcctgcagt tcgtggccga gaagtatggc ttcatcgacc 60

tgagccgagt tgccatccat ggctggctt acgggggctt cctctcgctc atggggctaa 120

tccacaagcc ccaggtgttc aaggtggcca tcgcgggtgc cccggtcacc gtctggatgg 180

cctacgacac agggtacacc tgagcgctac atggactgtc ctgagaacaa ccagcacggc 240

tatgaggcgg gtccgtggc cctgccacgt tgtgagaatg gctgccccca aatgagcccc 300

caaaccggc cttgccttt aatccntcc ccacgtggcg tttccctnt gtgttaaccg 360

aaaaaaacgt tgcaacactt atattttcg ccaagcgaag gccaaaaaca acggtttcc 420

ccaattacgg tgatcccttt ccccccaag aaccttggaa aattccccgg taatgcccag 480

gggggaaaaa ctttaccaag ctcccagatc taccggacg agagacacag tattcgctgc 540

cccgagtcgt ggcgagcact atgaagtca cttgtgcac ttttcctta acaaggggg 600

aaaatttaaa cccccctttttt ttggaaaggc ccccttggcc cccaa 645

<210> 26

<211> 448

<212> DNA

<213> Homo sapiens

<400> 26

gacataaaac ccaagagcgt ttaaaagaaaa ggataaaagg cgtcgaaaaa gtgaaggcag	60
cggttcctcg gggctggcca gctggggcg ggacaaagtgc cttcaactggg gccccgggc	120
cactcgtcc ctccccctg gttcccccg gaggctgcag ccacttgcgtc tgtgtatgtgg	180
cggtcccccgg tggcaggct cagaggtatt cctgttagaaa gtgcagcaac gtgacttcat	240
agtgtcgcc cgtactcgaaaa cagcgaataac tgtgtctctc gttggggtag atctggagct	300
ggtaaggttt cctgtcggtt atcagttggg agacgaggaa gtttgtgtgg aaaaagtgcac	360
cgttttcgcc caggaagccg tggaggataa gcaagcggtt ggctcattt ggcagcttc	420
ccacgtgcag gcccacggaa cccgcctc	448

<210> 27

<211> 442

<212> DNA

<213> Homo sapiens

<400> 27

gacataaaac ccaagagcgt ttaaaaaaag gataaaaggc gtcggggcg tgaaggcagc	60
ggcttcctcg ggcgtggccag cgctggcggt gacaaagtgc cttcaactggg cccggggcc	120
actcagtcac ttccccctgg ttcccccggtt aggtgcagc cacttgcgtc gtgtatgtggc	180
ggctcccggtt gggcaggctc agaggtattt cttgttagaaa tgcagcaac tgacttcata	240
gtgtcgcccc gactcgaaaa agcgaataact gtgtctctcg ttggggtaga tctggagctg	300
gttaaggtttt cctgtcggtt tcagttgggaa gacgaggaaat tttgtgtggaa aaaaagtgcac	360
gtttttcgcc aggaagccgtt ggaggataag caagcggtt ggctcattgg gcagcttc	420
cacgtgcagg gcccacggaaac cc	442

<210> 28

<211> 330

<212> DNA

<213> Homo sapiens

<400> 28

tctggtcgtg cgtggcatgg tgtggacttt gggtggtcg tgggcttctc cacgtgcagg	60
ccacggAACccgcctcatag ccgtgtcggt tggctcagt ggacgtccat gtagcgctca	120
gtgtacccctg tgctgttagtg ccatccagac ggtgaccggg gcacccgcga tggccacctt	180
gaacacctgg ggcttgtgga ttagccccat gagcgagagg aagccccgt aggaccagcc	240
atggatggca actcggtca ggtcgatgaa gccatacttc tggccacga actgcaggcc	300
ctccacctgg tcctcgatct ccaccatgag	330

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/01538

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7	C12N9/48	C12N15/12	C12N5/10	C12Q1/68	G01N33/50
	C12N15/62				

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EMBL, BIOSIS, EPO-Internal, WPI Data, PAJ, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>ABBOTT, CATHERINE A. ET AL: "Cloning, expression and chromosomal localization of a novel human dipeptidyl peptidase (DPP) IV homolog, DPP8" EUROPEAN JOURNAL OF BIOCHEMISTRY (2000), 267(20), 6140-6150, October 2000 (2000-10), XP002204858 cited in the application the whole document -& DATABASE EMBL 'Online' 6 November 2000 (2000-11-06) "Homo sapiens dipeptidyl peptidase 8 (DPP8) mRNA, complete cds" Database accession no. AF221634 XP002205003 61.6% identity over 2615 nt with SEQ ID No 1</p> <p>---</p> <p align="center">-/-</p>	<p>1-7, 10-12, 18-32, 34-51, 54-56, 61,64-67</p>

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the International filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the International filing date but later than the priority date claimed

- *T* later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the International search	Date of mailing of the International search report
19 July 2002	31/07/2002
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax (+31-70) 340-3018	Authorized officer Weikl, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/01538

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! 9 February 2001 (2001-02-09) "Mus musculus adult male testis cDNA, RIKEN full-length enriched library clone:4932434F09:homolog to Dipeptidyl peptidase 8, full insert sequence" Database accession no. AK016546 XP002204860 63.7% identity over 2499 nt with SEQ ID No 1 --- X DATABASE GENESEQ 'Online! 24 October 2000 (2000-10-24) "Human peptidase; HPEP-16; coding sequence" Database accession no. AAA37672 XP002205004 97.1% identity over 1520 nt with SEQ ID No 1 the whole document --- X DATABASE GENESEQ 'Online! 24 October 2000 (2000-10-24) "Human peptidase; HPEP-16; protein sequence" Database accession no. AAY90299 XP002205005 99.4% identity over 507 aa with SEQ ID No 2 the whole document --- X DATABASE GENESEQ 'Online! 8 February 2001 (2001-02-08) "Human ORFX ORF1390 polynucleotide sequence SEQ ID No 2779" Database accession no. AAC75835 XP002205006 95.3% identity over 1570 nt with SEQ ID No 1 the whole document --- X DATABASE GENESEQ 'Online! 8 February 2001 (2001-02-08) "Human ORFX ORF1390 polypeptide sequence SEQ ID No 2780" Database accession no. AAB41626 XP002205007 98.9% identity over 660 aa with SEQ ID No 2 the whole document ---	1-7, 10-12, 18-32, 34-51, 54-56, 61,64-67 1-7, 10-12, 30-32, 36-48 1-7, 10-12, 30-32, 36-48 1-7, 10-12, 30-32, 36-48 1-7, 10-12, 30-32, 36-48 1-7, 10-12, 30-32, 36-48

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/01538

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ABBOTT CATHERINE A ET AL: "Two highly conserved glutamic acid residues in the predicted beta propeller domain of dipeptidyl peptidase IV are required for its enzyme activity." FEBS LETTERS, vol. 458, no. 3, pages 278-284, XP004260275 ISSN: 0014-5793 figure 1 ---	1-7, 10-12, 18-32, 34-51, 54-56, 61,64-67
T	SEDO ALEKSI ET AL: "Dipeptidyl peptidase IV-like molecules: Homologous proteins or homologous activities?" BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1550, no. 2, December 2001 (2001-12), pages 107-116, XP002204859 ISSN: 0006-3002 table 1 ---	1-7, 10-12, 18-32, 34-51, 54-56, 61,64-67
P,X	WO 01 19866 A (ABBOTT CATHERINE ANNE ; GORELL MARK DOUGLAS (AU); UNIV SYDNEY (AU)) 22 March 2001 (2001-03-22) the whole document ---	1-7, 10-12, 18-32, 34-51, 54-56, 61,64-67

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claim 59 and claims 37, 46, 54, 55 and 56 (insofar as they relate to in vivo methods) are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition (in those cases where the compound/composition could be searched (see Box I.2)).

Continuation of Box I.2

Claims Nos.: 8, 9, 13, 14, 15, 16, 17, 33, 52, 53, 57, 58, 59, 60, 62, 63 and 68-71

Present claims 8, 9 (partially) 13, 14, 15 (partially) 16 (partially), 17 (partially) 33, 52, 53, 57, 58, 59, 60, 62, 63 and 68-71 relate to reagents defined by reference to a desirable characteristic or property, namely interaction with DPP8 polynucleotides or polypeptides or modulation of their activity.

The claims cover all products having this characteristic or property, whereas the application does not provide support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for any of these reagents. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the reagents by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has not been carried out for these claims (claims 15, 16 and 17 searched insofar as expression vector is concerned and claim 9 searched insofar as the methods of claims 6 or 7 are concerned).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/EP 02/01538**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 02/01538

Patent document cited in search report	Publication date		Patent family member(s)	Publication date
WO 0119866	A 22-03-2001	WO AU EP	0119866 A1 7394600 A 1214344 A1	22-03-2001 17-04-2001 19-06-2002